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Applicant	:	Komorowski, et al.
Appl. No.	:	10/090,038
Filed	:	February 27, 2002
For	:	CHROMIUM/BIOTIN TREATMENT OF DYSLIPIDEMIA AND DIET-INDUCED POST PRANDIAL HYPERGLYCEMIA
Examiner	:	Choi, Frank I.
Group Art Unit	:	1616

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, James Komorowski, declare and state as follows:

1. I am one of the named inventors on the above-captioned patent application, and am familiar with the contents of the patent application. I am the Vice President of Technical Services & Scientific Affairs at Nutrition 21, Inc., the assignee of the above-captioned patent application. The research at Nutrition 21, Inc. targets the metabolic diseases and disorders marketplace.

2. I have reviewed the above-captioned application in its entirety, including the specification and claims. I have also reviewed the contents of the file history of the above-captioned application including each of the Office Actions and all the references cited therein.

3. I received a Bachelor of Arts degree in biology from State University of New York and a M.S. in medical biology from Long Island University.

4. I have extensive experience in investigating the role of chromium, biotin, and other nutrients in ameliorating metabolic disorders, including, among others, insulin insensitivity. I joined Nutrition 21, Inc. in 1997. From 1997-1999, I served as Senior Manager of Clinical and Regulatory Affairs. In 1999, I became Director of Product Development, and in 2001 moved to my current position as Vice President of Technical Services and Scientific Affairs at Nutrition 21, Inc. Prior to my employ at Nutrition 21, Inc., I spent nine years in the pharmaceutical

industry, holding product management, clinical research, and regulatory affairs positions at major pharmaceutical companies. Additionally, I have worked for a number of years in academia as a research associate at both New York Hospital for Special Surgery and Memorial Sloan-Kettering Cancer Center.

5. Throughout the course of my career, I have completed more than 100 research studies, submitted over 10 regulatory submissions to the FDA, and am an inventor on over 12 patents. I lead Nutrition 21, Inc.'s scientific research team.

6. Since the 1990's I have worked in the field of metabolic diseases including type II diabetes and dyslipidemia. Several of the patents and patent applications on which I am a co-inventor relate to metabolic diseases including type II diabetes and dyslipidemia.

7. Many of the currently FDA approved drugs used for reducing hyperglycemia and stabilizing the levels of serum glucose are known to either not affect dyslipidemia or even make dyslipidemia worse, *e.g.*, by increasing LDL levels. Several studies have demonstrated inefficacy of anti-hyperglycemic drugs in treating dyslipidemia.

8. A meta-analysis by van Wijk et al. ((2003), *Arterioscler Thromb Vasc Biol.* 2003 23(10):1744-9)(Exhibit A), reports the effects of rosiglitazone and pioglitazone, two insulin sensitizing medications approved for the treatment of diabetes, for their effects on blood lipids *e.g.*, dyslipidemia. The analysis consisted of nineteen studies with 5,304 patients. Subjects receiving pioglitazone showed increased triglycerides and decreased HDL cholesterol levels. Further, subjects receiving higher dose of rosiglitazone (8 mg/d) showed a greater increase in total cholesterol and LDL cholesterol when compared to patients that received a lower dose (4 mg/d). These data illustrate that drugs that alleviate or treat diabetes do not necessarily alleviate or treat dyslipidemia.

9. Metformin is one of the most widely-used medications for reducing hyperglycemia. A study by Robinson et al. ((1998) Diabetes Care. May;21(5):701-5)(Exhibit B), analyzed metformin for its ability to improve glucose and lipid control. Robinson et al. report that metformin significantly improved glucose control, but had no significant effect on triglycerides or HDL cholesterol.

10. Samaha et al conducted a study in non-diabetic patients receiving rosiglitazone, an FDA approved drug for reducing hyperglycemia. Samaha et al. ((2006) Arterioscler Thromb Vasc Biol. 26(3):624-30)(Exhibit C) found that subjects receiving rosiglitazone had no significant change in HDL cholesterol levels and significantly increased total cholesterol levels. Fonseca et al. ((2000) JAMA. 283(13):1695-1702)(Exhibit E) conducted a study in diabetic patients receiving rosiglitazone and observed dose dependent increases in total and LDL cholesterol levels after rosiglitazone use.

11. The results of studies on the effects of different medications used for reducing hyperglycemia, such as those listed in ¶¶8-10, are summarized in a review by Clark et al. ((1998) Diabetes Spectrum, 11:4, pp. 211-221)(Exhibit D). Table 2 of Clark et al. lists several agents that can reduce hyperglycemia. In the row labeled "Plasma lipids in type 2 diabetes patients"

Appl. No. : **10/090,038**
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Clark et al. shows that the effect of hyperglycemic medications on plasma lipids ranges from positive, to no effect, to negative.

12. Exhibits A-E illustrate the understanding of those in the field of metabolic disorders that agents that reduce hyperglycemia and stabilize the levels of serum glucose will not necessarily or always also treat dyslipidemia and increase serum HDL cholesterol levels.

13. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

By: James Komorowski Date: June 7, 2006

James Komorowski

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EXHIBIT A

Thiazolidinediones and Blood Lipids in Type 2 Diabetes

Jeroen P.H. van Wijk, Eelco J.P. de Koning, Edwin P. Martens, Ton J. Rabelink

Abstract—We evaluated study population characteristics and treatment effects on blood lipids between studies in which either rosiglitazone (RSG) or pioglitazone (PIO) was investigated in patients with type 2 diabetes. We performed a summary analysis of all published double-blind, placebo-controlled studies with RSG (4 and 8 mg/d) and PIO (15, 30, and 45 mg/d). Data were analyzed by the random-effects model. Nineteen trials met our inclusion criteria, yielding 5304 patients, 3236 in studies with RSG and 2068 in studies with PIO. Subjects treated with PIO were more obese and showed more pronounced hyperglycemia and dyslipidemia (increased triglycerides and decreased HDL cholesterol) at baseline than did subjects treated with RSG. By weighted linear-regression analysis, studies with PIO showed greater beneficial effects on triglycerides, total cholesterol, and LDL cholesterol, after adjustment for the respective lipid levels at baseline. RSG 8 mg/d showed greater increases in total cholesterol and LDL cholesterol than did RSG 4 mg/d. PIO 30 mg/d showed greater reductions in triglycerides than did PIO 15 mg/d. Studies conducted with PIO showed more beneficial effects on blood lipids, but also different study population characteristics in comparison with studies conducted with RSG. Differences in both pharmacologic properties between agents and study population characteristics are likely to have influenced the results. (*Arterioscler Thromb Vasc Biol.* 2003;23:1744-1749.)

Key Words: thiazolidinediones ■ rosiglitazone ■ pioglitazone ■ lipids ■ cardiovascular disease

Thiazolidinediones (TZDs) are oral antihyperglycemic agents that reduce insulin resistance in peripheral tissues and decrease hepatic glucose production.[1] TZDs are potent, synthetic ligands for peroxisome proliferator-activated receptor gamma-γ (PPAR-γ) activation, which mediates the physiologic response by altering transcription of genes that regulate glucose and lipid metabolism.[2–4] Currently, there are 2 TZDs available: rosiglitazone (RSG) and pioglitazone (PIO). Troglitazone has been retracted from the market because of substantially increased risk of severe hepatotoxicity.[5–7] The clinical potency of TZDs is correlated closely with their PPAR-γ binding affinity. RSG has a greater PPAR-γ binding affinity than does PIO, which translates to a clinical dose that is ≈1/6th that of PIO.[4,8] Accordingly, the maximum recommended dose of 8 mg/d RSG corresponds to the maximum recommended dose of 45 mg/d PIO, whereas the submaximum dose of 4 mg/d RSG corresponds to the 30 mg/d submaximum dose of PIO.

The antihyperglycemic effects of RSG and PIO are well documented. RSG and PIO both demonstrate effective glycemic control when used as monotherapy or in combination with other antihyperglycemic agents.[9–12] TZDs also have important nonglycemic effects, such as modulation of lipid metabolism. It has been suggested that RSG and PIO differ in their effects on blood lipids and lipoproteins. Several studies have shown that treatment with PIO is associated with a greater beneficial effect on blood lipid levels than treatment with RSG.[13–16] Because dyslipidemia is an important risk factor for atherosclerosis, differential therapeutic modulation of lipid levels might confer a different level of protection from cardiovascular disease in patients with type 2 diabetes.

Several factors need to be considered when interpreting the effects of different TZDs on blood lipids. First, the differences between RSG and PIO might be related to specific pharmacologic properties of these agents. It has been shown that at the same clinical dose, PIO is associated with greater PPAR-α activation than is RSG.[17] PPAR-α is the main target for fibrates, a class of lipid-lowering drugs, which mainly reduce triglycerides (TGs) and increase HDL cholesterol (HDL-C).[18,19] Second, it is well recognized that the lipid-lowering responses of fibrates and statins are enhanced in patients with more pronounced dyslipidemia at baseline.[20,21] Baseline lipid levels might therefore influence the magnitude of treatment effects by TZDs.

We performed a summary analysis of all published double-blind, placebo-controlled studies to evaluate the effects of RSG and PIO on blood lipids in patients with type 2 diabetes. In addition, we critically evaluated study population characteristics between studies conducted with RSG and PIO.

Methods

Selection Criteria

We used PUBMED (http://www.ncbi.nih.gov/entrez/query.fcgi) to search the MEDLINE database up to December 2002 to identify all

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Arterioscler Thromb Vasc Biol. is available at http://www.atvbaha.org

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TABLE 1. General Characteristics of Studies With Rosiglitazone

Study	Journal, Year	Total Sample Size, No. (% females)	Monotherapy	Weight-Maintenance Diet	Mean Age, y	Treatment Dose, mg/d	Duration of Treatment, wk
Miyazaki et al[23]	*Diabetologia*, 2001	29 (45)	Yes	Yes	55.1	8	12
Raskin et al[22]	*Diabetes Care*, 2001	313 (44)	No	No	56.8	4 and 8	26
Lebovitz et al [11]	*J Clin Endocrinol Metab*, 2001	493 (34)	Yes	Yes	60.0	4 and 8	26
Phillips et al[25]	*Diabetes Care*, 2001	908 (37)	Yes	No	57.5	4 and 8	26
Raskin et al[24]	*Diabetologia*, 2000	208 (39)	Yes	No	58.5	4 and 8	8
Nolan et al[27]	*Diabet Med*, 2000	278 (38)	Yes	No	62.6	4 and 8	8
Wolffenbuttel et al[12]	*Diabet Med*, 2000	375 (44)	No	No	61.3	4	26
Gómez-Pérez et al[28]	*Diab Met Res Rev*, 2002	105 (74)	No	No	53.1	4 and 8	26
Fonseca et al[26]	*JAMA*, 2000	339 (32)	No	Yes	58.2	4 and 8	26
Patel et al[30]	*Diabetes Obes Metab*, 1999	155 (31)	Yes	Yes	58.9	4	12
Carey et al[31]	*Obes Res*, 2002	33 (18)	Yes	Yes	56.1	8	16
Pooled characteristics		3236 (39)	66%	34%	58.6	56% maximum dose	22

double-blind, randomized, placebo-controlled studies that evaluated the effects of RSG or PIO on blood lipids in patients with type 2 diabetes. The MEDLINE database was searched for the following terms: "rosiglitazone" and "pioglitazone." These searches were combined with searches for the terms "type 2 diabetes" and "placebo." The search was limited to English-language publications. No age or sex restriction was applied. Forty-six publications were identified by this search strategy. Subsequently, all full-text articles were reviewed, and studies were selected on the basis of a double-blind, placebo-controlled treatment period of at least 8 weeks with either RSG (doses of 4 and 8 mg/d) or PIO (doses of 15, 30, and 45 mg/d). Treatment effects on blood lipids should also have been reported by each study. Both TZD monotherapy and combination therapy with other antihyperglycemic agents (eg, sulfonylureas, metformin, or insulin) were considered eligible. Studies of combination therapy of TZD with lipid-lowering interventions (lipid-lowering agents or active lifestyle interventions) were excluded from analysis, although a concurrent weight-maintenance diet was considered acceptable when applied equally to all intervention arms.

Data Extraction

Both assessment of eligibility and data extraction were performed by a single, nonblinded reviewer (J.P.H.v.W.). The following information was extracted from each study: year of publication, sample size, sex distribution, participant age, TZD monotherapy or combination therapy, concurrent weight maintenance diet, duration of treatment with study medication, body mass index, and blood lipid levels (TGs, total cholesterol [TC], HDL-C, and LDL-cholesterol), including mean changes in each lipid parameter from baseline. Data extraction was performed for the RSG group, the PIO group, and the accompanying placebo groups (RSG placebo and PIO placebo).

Statistical Analysis

χ^2 tests were performed to test for heterogeneity of study results. Because the studies were conducted in various geographic areas, between-study variation could be expected. Hence, all data were combined by using the random-effects model of DerSimonian and Laird.[22] The random-effects model weights studies according to the sample size, the within-study variance, and the between-study variance. Weights were set equal to the reciprocal of the variance. We compared study population characteristics between different TZDs (RSG vs PIO) and between TZDs and placebo (RSG vs placebo and PIO vs placebo). In addition, we compared treatment effects on blood lipids (mean absolute changes from baseline) between RSG and PIO. We also performed a weighted linear-regression analysis for each lipid parameter (TGs, TC, HDL-C, and LDL-C) to compare the posttreatment blood lipid levels between studies in which either RSG or PIO was used, after adjustment for the respective lipid level at baseline. In this analysis, the posttreatment blood lipid level was used as the dependent variable and the baseline blood lipid level, as the independent variable. For statistical analysis, we used SPSS software, version 10.0 (SPSS Inc). Statistical significance was reached when $P<0.05$ (2 sided).

Results

Study Characteristics

Nineteen trials met our inclusion criteria, yielding 5304 patients: 3236 patients in studies with RSG (Table 1)[11,12,23–31] and 2068 patients in studies with PIO (Table 2).[9,10,32–37] RSG trials and PIO trials were comparable in sex distribution. Subjects in RSG trials were older than those in PIO trials. Sixty-six percent of the subjects in RSG trials received the study medication as monotherapy, whereas only 27% of the subjects did so in the PIO trials. A concurrent weight maintenance diet was more prevalent in PIO trials than in RSG trials (52% vs 34%, respectively). Fifty-six percent of the RSG group and 8% of the PIO group received the maximum recommended dose (8 mg/d for RSG and 45 mg/d for PIO, respectively). Fifty-seven percent received 30 mg/d PIO and 35%, 15 mg/d PIO. Mean duration of treatment was 22 weeks in the RSG trials and 18 weeks in the PIO trials.

Baseline Characteristics

The baseline characteristics of the RSG group, PIO group, and accompanying placebo groups (RSG placebo and PIO placebo) are shown in Table 3. Subjects in the PIO group were significantly younger and more obese than were those in the RSG group. In addition, subjects in the PIO group were characterized by a more pronounced hyperglycemia (increased fasting glucose and glycosylated hemoglobin) and dyslipidemia (increased TGs and decreased HDL-C) than in the RSG group. There were no differences in baseline characteristics between the TZDs and their accompanying placebo groups (RSG vs RSG placebo and PIO vs PIO placebo, respectively).

Treatment Effects of RSG and PIO

χ^2 tests revealed no statistical evidence of heterogeneity of study results (data not shown). The treatment effects of RSG

TABLE 2. General Characteristics of Studies With Pioglitazone

Study	Journal, Year	Total Sample Size, No. (% females)	Monotherapy	Weight-Maintenance Diet	Mean Age, y	Treatment Dose, mg/d	Duration of Treatment, wk
Miyazaki et al[32]	*Diabetes Care*, 2001	23 (26)	No	Yes	54.5	45	16
Kawamori et al[33]	*Diab Res Clin Pract*, 1998	30 (37)	No	No	54.6	30	12
Einhorn et al[34]	*Clin Ther*, 2000	328 (43)	No	Yes	55.6	30	16
Aronoff et al[9]	*Diabetes Care*, 2000	319 (42)	Yes	No	53.7	15, 30, and 45	26
Miyazaki et al[35]	*Diabetes Care*, 2002	45 (47)	Yes	No	54.7	15, 30, and 45	26
Rosenblatt et al[36]	*Coron Artery Dis*, 2001	197 (47)	Yes	Yes	54.5	30	16
Kipnes et al[10]	*Am J Med*, 2001	560 (41)	No	Yes	56.7	15, 30	16
Rosenstock et al[37]	*Int J Clin Pract*, 2002	566 (53)	No	No	57.1	15, 30	16
Pooled characteristics		2068 (45)	27%	52%	55.8	8% maximum dose	18

and PIO on blood lipids are shown in Figure 1. The treatment effects are shown as mean changes from baseline of TZD minus placebo for each lipid parameter (ΔRSG placebo and ΔPIO placebo, respectively). PIO was associated with significantly greater beneficial effects on all blood lipid levels.

Influence of Baseline Lipid Levels on Treatment Effects

Because subjects in studies with PIO were more dyslipidemic at baseline than were those in studies with RSG, we performed a weighted linear-regression analysis for each lipid parameter. From this analysis, posttreatment TGs (β=0.45, P<0.01), TC (β=0.56, P<0.001) and LDL-C (β=0.31, P<0.05) were higher in studies with RSG than in studies with PIO. However, posttreatment HDL-C was not significantly different between RSG and PIO (β=0.02, P=NS).

Treatment Effects of RSG and PIO per Treatment Dose

Treatment with the respective maximum recommended dose of each TZD was more prevalent in the RSG group than in the PIO group. Therefore, we performed a subgroup analysis in which we evaluated the effects of RSG and PIO on blood lipids per treatment dose (Table 4). The maximum and submaximum recommended doses of RSG (8 and 4 mg/d, respectively) had similar effects on TG and HDL-C. However, RSG at 8 mg/d was associated with significantly greater increases in TC and LDL-C compared with 4 mg/d RSG. PIO 30 mg/d was associated with significantly greater reductions in TG than was PIO 15 mg/d. The different doses of PIO had comparable effects on TC, HDL-C, and LDL-C.

Subgroup Analysis of Monotherapy Trials and Combination Therapy Trials

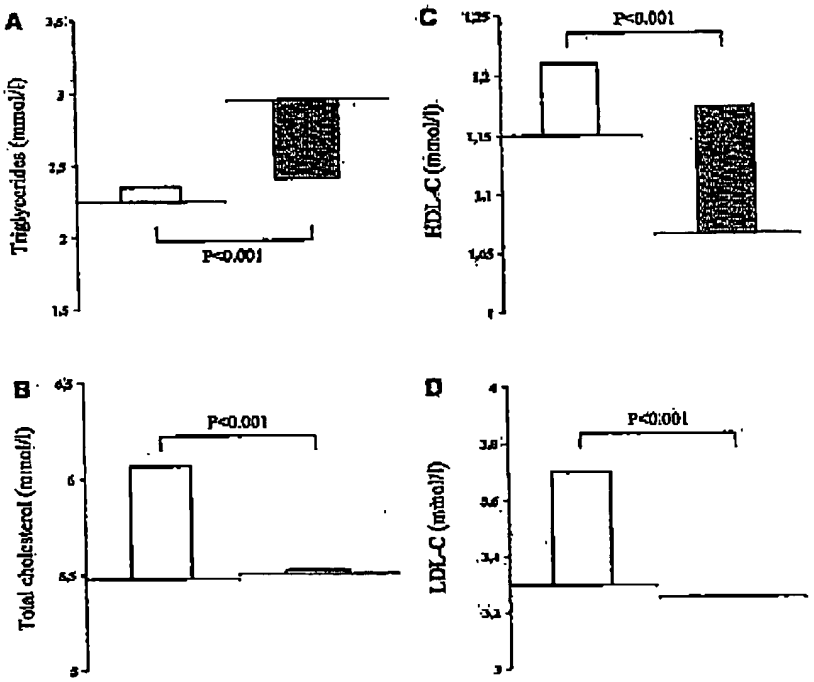
Because monotherapy was more prevalent in studies with RSG, we evaluated the treatment effects of RSG and PIO on blood lipids for monotherapy trials and combination therapy trials separately (Table 5). RSG combination therapy trials showed greater beneficial effects on all lipid levels than did RSG monotherapy trials. PIO combination therapy trials showed similar effects on blood lipids compared with PIO monotherapy trials. PIO monotherapy trials showed greater beneficial effects on all lipid levels compared with RSG monotherapy trials, whereas PIO combination therapy trials showed greater beneficial effects on TGs, TC, and LDL-C than did RSG combination therapy trials.

TABLE 3. Baseline Characteristics

	RSG Studies		PIO Studies	
	RSG-Group	RSG-Placebo	PIO-Group	PIO-Placebo
No. of patients	2194	1042	1328	740
Age, y	58.6 (0.2)*	58.9 (0.3)†	55.8 (0.27)	56.2 (0.35)
BMI, kg/m^2	29.7 (0.09)*	29.6 (0.13)†	32.2 (0.22)	32.0 (0.20)
Glucose, mmol/L	12.01 (0.07)*	11.63 (0.09)†	13.35 (0.10)	13.24 (0.14)
HbA_{1c}, %	9.0 (0.03)*	8.9 (0.05)†	9.8 (0.04)	9.8 (0.05)
Triglycerides, mmol/L	2.25 (0.04)*	2.09 (0.05)†	2.92 (0.08)	2.94 (0.11)
Cholesterol, mmol/L	5.48 (0.02)	5.46 (0.03)	5.49 (0.03)	5.70 (0.03)
HDL-C, mmol/L	1.15 (0.01)*	1.15 (0.01)†	1.07 (0.01)	1.08 (0.01)
LDL-C, mmol/L	3.30 (0.02)	3.31 (0.03)	3.25 (0.03)	3.28 (0.04)

Data are mean (SE). All data were analyzed by using the random-effects model.

In this analysis, comparisons between different TZD (RSG vs PIO) and between TZD and placebo (RSG vs placebo and PIO vs placebo) were performed. *P<0.001 vs PIO-group; †P<0.001 vs PIO-placebo.



Mean treatment effects of TZDs minus the mean treatment effect of the accompanying placebo for each lipid parameter: TGs (a), TC (b), and HDL-C (c). White bars indicate RSG; black bars, PIO.

Discussion

In clinical practice, there is much debate concerning the potential different effects of RSG and PIO on blood lipids. This might have important implications, because dyslipidemia is a major risk factor for atherosclerosis in patients with type 2 diabetes. Because no data on prospective, randomized, double-blind PIO versus RSG studies were available, we performed a summary analysis of all published double-blind, placebo-controlled studies with either RSG or PIO. The main outcome of our summary analysis is that studies with PIO showed more beneficial treatment effects on blood lipids in comparison with studies with RSG, but important differences in baseline characteristics existed between the study populations.

During the past several years, TZDs have received increasing attention for treatment of patients with type 2 diabetes. The antihyperglycemic effects of RSG and PIO are well documented and appear to be equivalent between comparable doses of the 2 agents.[13] In addition to glucose lowering, TZDs influence lipid metabolism, most likely by directing a PPAR-γ–mediated change in adipocyte metabolism and insulin sensitivity. Hence, TZDs could potentially modulate the characteristic diabetic dyslipidemia, which is characterized by increased TGs, reduced HDL-C and the predominance of atherogenic, small, dense LDL particles.[28]

TABLE 4. Treatment Effects of RSG and PIO Per Treatment Dose

	RSG 4 mg/d	RSG 8 mg/d	
ΔTriglycerides, mmol/L	+0.13 (0.06)	+0.05 (0.07)	
ΔCholesterol, mmol/L	+0.52 (0.04)*	+0.70 (0.04)	
ΔHDL-C, mmol/L	+0.05 (0.01)	+0.06 (0.01)	
ΔLDL-C, mmol/L	+0.34 (0.03)*	+0.48 (0.04)	
	PIO 15 mg/d	PIO 30 mg/d	PIO 45 mg/d
ΔTriglycerides, mmol/L	−0.44 (0.08)†	−0.66 (0.07)	−0.38 (0.16)
ΔCholesterol, mmol/L	−0.01 (0.06)	+0.01 (0.05)	+0.10 (0.15)
ΔHDL-C, mmol/L	+0.10 (0.02)	+0.09 (0.02)	+0.11 (0.04)
ΔLDL-C, mmol/L	+0.08 (0.06)	−0.01 (0.04)	+0.15 (0.12)

ΔTriglycerides, ΔCholesterol, ΔHDL-C, and ΔLDL-C is the difference in concentration for triglyceride, total cholesterol, HDL-C and LDL-C, respectively, between the active treatment group and placebo group for each specific TZD dose. Data are mean (SE).

*$P<0.05$ vs RSG 8 mg/d. †$P<0.05$ vs PIO 30 mg/d.

TABLE 5. Treatment Effects of RSG and PIO for Monotherapy Trials and Combination Therapy Trials

	Monotherapy	Combination Therapy
Rosiglitazone trials		
ΔTriglycerides, mmol/L	+0.21 (0.06)*	−0.06 (0.07)
ΔCholesterol, mmol/L	+0.68 (0.03)*	+0.46 (0.05)
ΔHDL-C, mmol/L	+0.03 (0.01)*	+0.11 (0.01)
ΔLDL-C, mmol/L	+0.43 (0.03)*	+0.33 (0.04)
Pioglitazone trials		
ΔTriglycerides, mmol/L	−0.51 (0.09)†	−0.57 (0.06)†
ΔCholesterol, mmol/L	+0.05 (0.07)†	−0.01 (0.05)†
ΔHDL-C, mmol/L	+0.09 (0.02)†	+0.10 (0.01)
ΔLDL-C, mmol/L	+0.07 (0.06)†	+0.02 (0.04)†

Data are mean (SE).

*$P<0.05$ vs combination therapy. †$P<0.05$ vs RSG trials.

We found that studies with PIO show greater beneficial effects on TGs, TC, and LDL-C than did studies with RSG. Whether the magnitude of these differences is sufficient to produce clinically relevant cardiovascular benefits is an open question. The currently available data support a dose-dependent effect of RSG on TC and LDL-C, whereas PIO might exert dose-related effects on TGs. However, only a small number of subjects were receiving the maximum recommended dose of PIO.

Studies with PIO showed greater beneficial effects on TGs than did studies with RSG. Several factors might explain the differential effects of RSG and PIO on TG levels. First, it has been shown that at the same clinical dose, PIO is associated with greater PPAR-α activation than is RSG.[17] PPAR-α is the main target for fibrates, a class of lipid-lowering drugs, which mainly reduce TGs and increase HDL-C.[18,19] Increased PPAR-α activation by PIO might explain the observed beneficial effects of PIO on TGs. Second, it is well recognized that the lipid-lowering responses are partly dependent on the baseline characteristics of the study group. The lipid-lowering responses of fibrates and statins are enhanced in patients with more pronounced dyslipidemia at baseline.[20,21] In our summary analysis, we have shown that subjects treated with PIO were characterized by a more pronounced dyslipidemia (increased TGs and decreased HDL-C) at baseline than were those treated with RSG. These differences in patient baseline characteristics between studies with RSG and PIO are likely to have influenced the magnitude of the effects on TGs and HDL-C. The observation that after adjustment for baseline HDL-C, there was no longer a statistically significant difference in posttreatment HDL-C between RSG and PIO supports this hypothesis. Moreover, in a recent study with PIO, it was shown that patients with the lowest baseline HDL-C levels responded with HDL-C increases of greater magnitude than did those who had higher HDL-C levels at baseline.[36] Studies with RSG showed greater increases in TC and LDL-C compared with studies with PIO, despite similar baseline levels. Why RSG and PIO exert different effects on TC and LDL-C is an open issue. Interestingly, TZDs improve LDL particle density, causing a shift from small, dense LDL particles to larger, buoyant LDL particles, which are less prone to oxidative modification and are therefore, thought to be less atherogenic.[39–42] These changes in LDL-C density elicited by TZDs might be more meaningful than the small changes in overall LDL-C levels.

Besides differences in baseline lipids, subjects treated with PIO were more obese and had worse glycemic control at baseline than did subjects treated with RSG. In addition, a concurrent weight maintenance diet was more prevalent in PIO trials than in RSG trials, whereas more subjects in RSG trials were on monotherapy. These factors might also have influenced the results. Interestingly, RSG combination therapy trials showed greater beneficial effects on all blood lipids compared with RSG monotherapy trials. These differences were not observed in studies with PIO. Because monotherapy was more prevalent in RSG trials, this could have contributed to the results. Regrettably, the number of studies was limited, and we could not adjust for other relevant parameters (eg, body mass index, glycemic control) to more reliably estimate the differences in treatment effects between studies with RSG and those with PIO. Although differences in study population characteristics were a confounding factor for our analysis, it should be noted that this is also one of the most interesting findings that is often not accounted for when discussing differential effects of TZDs in clinical practice. Apparently, studies with RSG are performed in a "different patient population" than are studies with PIO. Our results emphasize the importance of study population characteristics when examining clinical data from studies performed with different TZDs. Clearly, there is a need for direct, double-blind comparisons of the 2 agents in the same population.

Our data are in line with several open-label, prospective or retrospective studies on the effects of RSG and PIO on blood lipids.[14,15] Khan et al[14] performed an open-label, randomized comparison of RSG and PIO in patients previously treated with troglitazone. In that study, conversion to pioglitazone was associated with significant improvements in all lipid levels, whereas conversion to RSG led to significant increases in all lipid levels, despite similar weight increases and glycemic control in the RSG group and PIO group. In a recent retrospective review of randomly selected medical records, it was shown that treatment with PIO was associated with greater beneficial effects on blood lipid levels than was treatment with RSG, despite similar glycemic control.[15] However, that article failed to take into account a large body of evidence from double-blind, randomized, placebo-controlled studies, which represent the "gold standard" for clinical analysis.

In conclusion, studies conducted with PIO showed more beneficial effects on blood lipids, but also different study population characteristics, in comparison with studies conducted with RSG. Differences in both pharmacologic properties and study population characteristics between the 2 agents are likely to have influenced the results. When examining the available clinical data from studies performed with different TZDs, it is important to interpret the results in light of the prevailing study population characteristics.

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Clinical Care/Education/Nutrition
ORIGINAL ARTICLE

EXHIBIT B

The Effects of Metformin on Glycemic Control and Serum Lipids in Insulin-Treated NIDDM Patients With Suboptimal Metabolic Control

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OBJECTIVE— To test the hypothesis that metformin therapy, given as an adjunct to insulin therapy, improves metabolic control in insulin-treated NIDDM patients with suboptimal glycemic control.

RESEARCH DESIGN AND METHODS— A total of 33 subjects with insulin-treated NIDDM were investigated; all had commenced insulin after secondary failure of antihyperglycemic agents. Two randomized double-blind placebo-controlled crossover studies were run. In study 1 (n = 19), insulin-treated subjects with suboptimal glycemic control received 12 weeks of metformin 1 g b.i.d. and 12 weeks of placebo. In study 2 (n = 14), subjects already established on adjunctive metformin/insulin therapy stopped the metformin component and received 12 weeks of metformin at their baseline dosage (range 1–2.5 g) and 12 weeks of equivalent placebo. Fasting plasma glucose, HbA_{1c}, and serum lipids were measured at baseline and midway through and at the end of each treatment phase. The effect of 12 weeks of metformin treatment was compared with the effect of 12 weeks of placebo in each study and in both studies combined.

RESULTS— In study 1, metformin treatment was associated with significant improvements in fasting plasma glucose (mean 12-week difference from placebo [95% CI]: 5.8 mmol/l [3.5–8.1], $P < 0.001$) and HbA_{1c} (1.6% [0.9–2.4], $P < 0.001$). In study 2, metformin treatment was associated with significantly lower fasting plasma glucose (5.3 mmol/l [0.6–9.9], $P = 0.029$) and lower HbA_{1c} (2.4% [1.0–3.8], $P = 0.003$) compared with those for placebo. Study 2 also showed metformin treatment to be associated with significantly lower total cholesterol than that for placebo (1.0 mmol/l [0.1–1.9], $P = 0.032$) and lower LDL cholesterol (1.0 mmol/l [0.1–1.9], $P = 0.028$). This significant difference in serum lipids seen in study 2 was not seen in study 1, but was present when both sets of data were combined (n = 33, mean total cholesterol difference at 12 weeks [95% CI]: 0.6 mmol/l [0.1–1.1], $P = 0.015$). Metformin had no significant effect on triglyceride, HDL cholesterol, weight, or blood pressure. Two subjects on metformin withdrew because of side effects.

CONCLUSIONS— Metformin, when given as adjunctive therapy, was well tolerated and improved glycemic control and lipid concentrations in patients with insulin-treated NIDDM whose diabetes was poorly controlled. These improvements could be maintained over the long term.

Metformin has been used in the U.K. since 1957, particularly for overweight patients with NIDDM, and has recently been approved for use in the U.S. (1). It has a significant antihyperglycemic action and a beneficial effect on serum lipids (2,3). It has been shown to lower both total and LDL cholesterol and serum triglycerides in NIDDM (4,5). Many studies have shown a significant association of metformin treatment with weight loss (6). The underlying mechanisms for the drug's antidiabetic effects are not fully understood, but it is accepted that they are not mediated through increased insulin secretion. Identified mechanisms include suppression of hepatic glucose output and an increase in peripheral glucose uptake and intestinal glucose use (7). Thus, we hypothesized that metformin was a suitable drug for combination with insulin in the treatment of poorly controlled insulin-treated NIDDM. We aimed to study the effects of giving metformin to insulin-treated NIDDM subjects with suboptimal metabolic control in two randomized placebo-controlled crossover studies.

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RESEARCH DESIGN AND METHODS

Study 1

The study was approved by the local research ethics committee of the Kensington, Chelsea, and Westminster Health Authority. Patients with NIDDM were selected from a teaching hospital outpatient diabetic clinic. The diagnosis of NIDDM was based on clinical history and the finding of a fasting plasma glucose concentration >7.8 mmol/l on two occasions. In all cases, insulin had been started after secondary failure of maximum-dose oral antihyperglycemic agents and had been their sole diabetic treatment for at least 1 year. Female subjects of childbearing age, those unable to give fully informed consent, and those already taking any oral antihyperglycemic agent in addition to their insulin were excluded. There was no upper age limit. All patients entered a 6-week run-in phase with two baseline assessments (on days 1 and 28) to determine eligibility for the randomized treatment phase. At enrollment, demographic details and a diabetic history were obtained with a fasting blood sample for laboratory measurements of glucose, creatinine, HbA_{1c}, total cholesterol,

Table 1—*Baseline characteristics of the patients*

	Study 1	Study 2
n	19	14
Age (years)	61.3 ± 7.1	56.1 ± 8.9
Sex (M/F)	7/12	3/11
Weight (kg)	80.9 ± 6.9	83.2 ± 12.7
BMI (kg/m²)	29.5 ± 3.5	30.9 ± 3.8
Duration of diabetes (years)	15 ± 7	14 ± 6
Retinopathy (yes/no)*	9/10	9/5
Neuropathy (yes/no)*	6/13	3/11
Proteinuria (yes/no)†	1/18	4/10
Systolic blood pressure (mmHg)	137 ± 16	144 ± 23
Diastolic blood pressure (mmHg)	79 ± 10	87 ± 11
Metformin dose (g/24 h)	—	2.0 ± 0.5
Insulin requirement (U/day)	71 ± 47	41 ± 16

Data are *n* or means ± SD. *Information from review of clinical notes. †On dipstick testing.

triglycerides, and HDL cholesterol. On the second baseline visit, a fasting blood sample was taken for glucose and HbA_{1c} measurement, and any change in insulin requirement was noted. Inclusion criteria necessary to proceed to the treatment phase were as follows: suboptimal glycemic control (HbA_{1c} ≥7.5%), stable glycemic control and insulin dosage (baseline values to differ by ≤15%), normal renal function (creatinine ≤125 μmol/l), and BMI ≥23 kg/m².

There were 20 qualifying subjects randomized to receive either metformin 500 mg b.i.d. (increasing to 1 g b.i.d. after 7 days) or placebo for 12 weeks, at which point crossover took place, the treatment phases lasting a total of 24 weeks.

Assessments were performed at 6-week intervals. Each subject attended the metabolic investigation unit at St. Mary's Hospital between 8:00 A.M. and 10:00 A.M. after a 12-h fast. Adverse effects were detected by direct questioning. After examination of the patient's general condition, weight and height were recorded in light clothing and without shoes; resting blood pressure was recorded in the right arm with diastolic pressure recorded at phase V Korotkoff sounds. Venous blood was analyzed for glucose, HbA_{1c}, total cholesterol, triglycerides, and HDL cholesterol. At the conclusion of each visit, subjects were given metformin or matching placebo (Lipha Pharmaceuticals, West Drayton, U.K.), and returned tablets were counted. We aimed to keep insulin dosage constant for the duration of the treatment phase with adjustments made only in the presence of hypoglycemia or significant hyperglycemic symptoms.

Study 2

The study was approved by the local research ethics committee of the Barnet Health Authority. Subjects with NIDDM (diagnostic criteria as for study 1) were selected from a district general hospital outpatient diabetic clinic. As in study 1, all patients were insulin-treated after secondary failure of maximum-dose oral antihyperglycemic agents. In contrast to those entering study 1, however, these subjects were already taking metformin as the sole oral antihyperglycemic agent in addition to their insulin therapy (1,000–2,550 mg/day) and had been doing so for at least 1 year (range 2–11 years). Metformin had been combined with insulin because of suboptimal glycemic control when using insulin alone, despite steady increases in daily insulin dosage. Other inclusion and exclusion criteria were the same as for those of study 1, and all entered an identical run-in phase. The entry criteria for the treatment phase were as follows: stable glycemic control and insulin dosage (baseline values to differ by ≤15%), normal renal function (creatinine ≤125 μmol/l), and BMI ≥23 kg/m².

There were 15 qualifying subjects randomized to receive either metformin (500- or 850-mg tablets) or matching placebo in a regimen equivalent to their normal metformin prescription, which was stopped. At 12 weeks, crossover took place, the treatment phase lasting a total of 24 weeks.

Assessments were performed at 6-week intervals, following the same protocol as in study 1. At the conclusion of each visit, metformin or matching placebo was dispensed, and returned tablets were counted. Again, we aimed to keep insulin dosage constant for the duration of the treatment phases with adjustments made only in the presence of hypoglycemia or significant hyperglycemic symptoms.

Analytic methods

Plasma glucose, triglycerides, total cholesterol, and HDL cholesterol were quantified by enzymatic techniques using an Olympus AU5200 analyzer (Olympus). HbA_{1c} was quantified after separation by low-pressure cation exchange chromatography in conjunction with gradient elusion using a 765 Glycomat analyzer. LDL cholesterol was calculated using Friedewald's formula (8).

Statistical analysis

Data are expressed as means ± SD. To assess the differences between the two treatments, we compared the change in variables over the 12 weeks of each of the two treatment phases: the metformin phase or the placebo phase. Changes in variables have been calculated as values at the end of a 12-week phase minus values at the beginning of that 12-week phase. For triglycerides, changes in values refer to ratios: end of period to start of period. Differences between the changes in variables after metformin and placebo treatment were analyzed using the method for a two-way crossover clinical trial described by Hills and Armitage (9), which takes into account any differences due to order of treatment (first or second phase) and also any carryover effects from one treatment phase to the next. Means and 95% CIs for the differences between treatments are given.

RESULTS

Study 1

We recruited 20 subjects, and 19 completed the study. One subject withdrew after the onset of diarrhea early in the metformin phase. Baseline characteristics of the remaining 19 subjects are shown in Table 1. One subject suffered mild abdominal bloating and completed the study on a reduced dose of metformin (500 mg b.i.d.). One subject suffered from a VIth nerve palsy during the placebo phase; investigation concluded that this was a complication of his diabetes. All exhibited poor but stable glycemic control (mean of baseline HbA_{1c} 9.1 ± 1.2% [normal range <6.5%]; mean difference between first and second baseline HbA_{1c} 5.4 ± 4.5% of first value). Insulin dosage was also stable during the

Table 2—*Results from study 1: effects of 12-week metformin versus placebo on clinical and metabolic parameters*

Parameter	Baseline	Change after 12 weeks placebo	Change after 12 weeks metformin	Difference	*P* value
Body weight (kg)	81.1 ± 16.9	0.0 ± 1.8	−0.5 ± 3.1	0.5 (−1.0 to 2.1)	0.465
Fasting plasma glucose (mmol/l)	11.8 ± 3.7	1.9 ± 3.8	−3.8 ± 3.2	5.8 (3.5 to 8.1)	<0.001
HbA_{1c} (%)	8.9 ± 1.0	0.5 ± 0.9	−1.1 ± 1.3	1.6 (0.9 to 2.4)	<0.001
Total cholesterol (mmol/l)	6.0 ± 1.1	0.0 ± 0.8	−0.3 ± 0.7	0.3 (−0.2 to 0.8)	0.248
HDL cholesterol (mmol/l)	1.1 ± 0.3	0.1 ± 0.2	0.0 ± 0.1	0.1 (−0.1 to 0.2)	0.284
Triglycerides (mmol/l)	2.2 ± 1.3	1.0 ± 0.4	0.9 ± 0.2	0.1 (−0.2 to 0.3)	0.543
LDL cholesterol (mmol/l)	3.9 ± 1.2	0.0 ± 0.8	−0.2 ± 0.6	0.3 (−0.2 to 0.7)	0.244
Systolic blood pressure (mmHg)	138 ± 16	2 ± 23	−4.0 ± 13	6 (−11 to 23)	0.452
Diastolic blood pressure (mmHg)	78 ± 9	4 ± 11	−3 ± 9	7 (0 to 15)	0.053

Data are means ± SD or means (95% CI). Change was calculated as the value at the start of each 12-week treatment period (metformin or placebo) minus the value at the end of that 12-week treatment period; a negative value implies a lowering of that value. The difference was calculated by subtracting change after metformin from change after placebo (weighted according to the number of patients receiving metformin first and the number receiving placebo first). For triglycerides, changes refer to ratios (end of period to start of period). *P* values were assessed using methods described by Hills and Armitage.

run-in phase, with only one subject altering his or her total daily dose (4-U increase). The results of study 1 are shown in Table 2. There was no evidence of a treatment-order or carryover effect. Daily insulin dosage was relatively constant throughout the treatment phase: mean change during the placebo phase was +0.6 U and during the metformin phase −1.9 U. Metformin treatment was associated with significant improvement in fasting plasma glucose at the 12-week assessment (mean difference from placebo 5.8 mmol/l, $P < 0.001$). Similarly, metformin treatment was associated with significant improvement in HbA_{1c} at 12 weeks (mean difference from placebo 1.6%, $P < 0.001$). Insignificant numerical improvements in both HbA_{1c} and fasting plasma glucose were seen after 6 weeks of metformin treatment. There were no significant changes in weight, total cholesterol, triglycerides, HDL cholesterol, or diastolic and systolic blood pressure. There were no significant correlations between change in BMI and change in fasting plasma glucose or HbA_{1c}. No serious hypoglycemic episodes were reported, and compliance was satisfactory as assessed by tablet counting.

Study 2

We recruited 15 subjects, and 14 satisfactorily completed the study. One subject withdrew complaining of abdominal pain and gastric upset. She recovered, and her symptoms were judged to be related to a past history of diverticular disease. Baseline characteristics of the subjects who completed the treatment phase are shown in Table 1. All exhibited stable glycemic control (mean difference between first and second baseline HbA_{1c} 5.8 ± 4.7% of first value). Insulin dosage was also stable during the run-in phase, with only three subjects altering their daily total, and each by just 2 U. The results of study 2 are shown in Table 3. There was no evidence of a treatment-order or carryover effect. While total daily insulin dosage remained relatively constant during the metformin phase (mean change −0.2 U), it tended to be increased during the placebo phase to combat symptomatic hyperglycemia (mean increase 9.0 U). Despite this, metformin treatment, as compared with placebo, was associated with significantly lower fasting plasma glucose values at the 12-week assessments (mean difference 5.3 mmol/l, $P = 0.029$) and with significantly lower HbA_{1c} (mean difference 2.4%, $P = 0.003$). Metformin treatment was also associated with lower total cholesterol values at 12 weeks (mean difference from placebo 1.0 mmol/l, $P = 0.032$) and also

Table 3—*Results from study 2: effects of 12-week metformin versus placebo on clinical and metabolic parameters*

Parameter	Baseline	Change after 12 weeks placebo	Change after 12 weeks metformin	Difference	*P* value
Body weight (kg)	83.2 ± 12.7	−0.8 ± 1.6	0.3 ± 2.2	−1.0 (−2.2 to 0.3)	0.116
Fasting plasma glucose (mmol/l)	10.6 ± 4.2	2.5 ± 3.0	−3.0 ± 6.1	5.3 (0.6 to 9.9)	0.029*
HbA_{1c} (%)	9.5 ± 1.2	1.4 ± 1.5	−1.1 ± 1.3	2.4 (1.0 to 3.8)	0.003†
Total cholesterol (mmol/l)	6.4 ± 1.2	0.5 ± 0.6	−0.6 ± 1.1	1.0 (0.1 to 1.9)	0.032†
HDL cholesterol (mmol/l)	1.2 ± 0.4	0.0 ± 0.3	0.1 ± 0.2	−0.1 (−0.4 to 0.2)	0.439
Triglycerides (mmol/l)	2.5 ± 2.4	1.1 ± 0.3	1.1 ± 0.2	0.1 (−0.2 to 0.3)	0.665
LDL cholesterol (mmol/l)	4.1 ± 1.5	0.4 ± 0.6	−0.7 ± 1.0	1.0 (0.1 to 1.9)	0.028†
Systolic blood pressure (mmHg)	144 ± 23	3 ± 24	1 ±18	0 (−21 to 21)	0.985
Diastolic blood pressure (mmHg)	87 ± 11	−1 ± 9	3 ± 9	−6 (−14 to 2)	0.135

Data are means ± SD or means (95% CI). Change was calculated as the value at the start of each 12-week treatment period (metformin or placebo) minus the value at the end of that 12-week treatment period; a negative value implies a lowering of that value. The difference was calculated by subtracting change after metformin from change after placebo (weighted according to the number of patients receiving metformin first and the number receiving placebo first). For triglycerides, changes refer to ratios (end of period to start of period). *P* values were assessed using methods described by Hills and Armitage. *$P < 0.05$; †$P < 0.01$.

with lower LDL cholesterol values (mean difference from placebo 1.0 mmol/l, $P = 0.028$). Insignificant numerical improvements in both HbA_{1c}, fasting plasma glucose, and total and LDL cholesterol were seen after 6 weeks of metformin treatment. There were no significant changes in weight, triglycerides, HDL cholesterol, and diastolic or systolic blood pressure. There were no significant correlations between change in BMI and change in fasting plasma glucose, HbA_{1c}, and LDL or total cholesterol. No serious hypoglycemic episodes were reported and compliance was considered satisfactory.

Studies 1 and 2 combined

When both sets of data were analyzed together, metformin-associated improvements in fasting plasma glucose, HbA_{1c}, and total and LDL cholesterol remained significant (mean differences: fasting plasma glucose 5.6 mmol/l, $P < 0.001$; HbA_{1c} 2.0%, $P < 0.001$; total cholesterol 0.6 mmol/l, $P = 0.015$; LDL cholesterol 0.6 mmol/l, $P = 0.015$).

CONCLUSIONS— Lifestyle modifications are first-line treatment for NIDDM patients. However, only a minority achieve satisfactory metabolic control after 1 year. Second-line treatment is with oral antihyperglycemic agents, usually a sulfonylurea, with metformin reserved for the obese. Upon failure of oral antihyperglycemic agents, conversion to insulin therapy is the usual next treatment step. Many insulin-treated NIDDM patients still achieve only poor glycemic control and suffer high complication rates (10–13). Despite progressive increases in dosage, insulin may not improve diabetic control and often causes weight gain. NIDDM patients who are insulin-treated subsequent to oral antihyperglycemic agent failure, who also exhibit weight gain and poor metabolic control despite increasing insulin dosage, can usefully be described as having insulin treatment failure.

There is no consensus on how best to treat poorly controlled insulin-treated NIDDM patients. Options include intensifying the insulin regimen or combining insulin with an oral antihyperglycemic agent. The Veterans Affairs Cooperative Study on Glycemic Control and Complications in Type II Diabetes showed how a four-step intensive therapy regimen could substantially improve glycemic control, as compared with a single morning insulin injection regimen (14). However, most of the decrease in HbA_{1c} occurred with a single dose of intermediate insulin at bedtime; the addition of glipizide and multiple daily insulin injections led to only small additional decreases in HbA_{1c}. Some studies have shown significant improvements in glycemic control and serum lipids when sulfonylureas are given in addition to insulin (15) but have failed to show a fall in serum insulin levels (16), despite reductions in exogenous insulin dosage, or a reduction in weight, and none have investigated long-term effectiveness.

There are few data on the adjunctive use of metformin. Metformin has been shown to be safe and effective in improving glycemic control in NIDDM patients when diet or sulfonylureas alone have been inadequate (1,17,18) and, in just one study, in obese insulin-treated NIDDM subjects (19). In addition, reductions in weight, triglyceride, LDL cholesterol, insulin resistance, and plasminogen activator inhibitor and an increase in HDL cholesterol (2–5,20–23) have been recorded. The current studies found that adjunctive metformin therapy significantly improves glycemic control in NIDDM patients who have insulin treatment failure, reducing fasting plasma glucose by 40% and HbA_{1c} by 20%. LDL cholesterol was lowered by 15%. There was no apparent change in HDL cholesterol, in contrast to some previous studies where an increase has been associated with metformin therapy (3,24). Long-term benefit from metformin is suggested by our study 2 data, where cessation of additional metformin therapy in insulin-treated NIDDM resulted in a deterioration of glycemic control and LDL cholesterol despite increases in total daily insulin dosage. The reduction in LDL cholesterol associated with metformin therapy in our studies is of interest because of the strong association between LDL cholesterol concentration and the development of ischemic heart disease and the demonstration that a reduction in LDL cholesterol is associated with a reduction of coronary mortality in nondiabetic men (25). Metformin had no effect on weight in either study 1 or 2, in contrast with several previous sulfonylurea and insulin combination studies (26,27).

In conclusion, adjunctive metformin therapy may improve metabolic control in insulin-treated NIDDM patients.

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EXHIBIT C

Effects of Rosiglitazone on Lipids, Adipokines, and Inflammatory Markers in Nondiabetic Patients With Low High-Density Lipoprotein Cholesterol and Metabolic Syndrome

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Background—PPAR-γ agonists improve insulin sensitivity and glycemic control in type 2 diabetes and may reduce atherosclerosis progression. Thus, PPAR-γ agonists may be an effective therapy for metabolic syndrome. However, the full spectrum of potentially antiatherogenic mechanisms of PPAR-γ agonists have not been fully tested in nondiabetic patients with metabolic syndrome.

Methods and Results—We performed a prospective, double-blinded, placebo-controlled study of 60 nondiabetic subjects with low high-density lipoprotein cholesterol (HDL-C) level and metabolic syndrome to rosiglitazone 8 mg daily or placebo for 12 weeks. We found no significant effect of rosiglitazone on HDL-C (+5.5% versus +5.8%, $P=0.89$), and an increase in total cholesterol (+8% versus −1%; $P=0.03$). Nevertheless, rosiglitazone significantly increased adiponectin (+168% versus +25%; $P<0.001$), and lowered resistin (−6% versus +4%; $P=0.009$), C-reactive protein (−32% versus +36%, $P=0.002$), interleukin (IL)-6 (−22% versus +4%, $P<0.001$), and soluble tumor-necrosis factor-α receptor-2 (−5% versus +7%, $P<0.001$).

Conclusions—These findings suggest that rosiglitazone, presumably through its PPAR-γ agonist properties, has direct effects on inflammatory markers and adipokines in the absence of favorable lipid effects. These findings may help explain the mechanism underlying the possible antiatherosclerotic effects of rosiglitazone. (*Arterioscler Thromb Vasc Biol.* 2006;26:624-630.)

Key Words: adipocytokines ■ lipids ■ inflammation ■ lipoprotein metabolism ■ arteriosclerosis

The metabolic syndrome is characterized by depressed high-density lipoprotein-cholesterol (HDL-C), elevated triglycerides, central obesity, impaired glucose tolerance, and elevated blood pressure.[1] These clinical factors underlie an overall insulin resistant and pro-inflammatory state. Approximately 44% of the US population older than age 50 years has the metabolic syndrome,[2] which is of concern, because it is associated with a 60% higher prevalence of coronary heart disease.[2]

Synthetic peroxisome proliferator-activated receptor (PPAR)-γ agonists, or thiazolidinediones (TZDs), including rosiglitazone and pioglitazone, improve insulin sensitivity, at least partly through PPAR-γ activation in adipose tissue,[3] and improve glycemic control in type 2 diabetes. Rosiglitazone raises HDL-C levels from 3% to 13.6%,[4–9] and lowers plasma CRP levels[10] in patients with type 2 diabetes. Thus, in patients with diabetes, rosiglitazone has a variety of effects that might reduce cardiovascular risk. Rosiglitazone has been shown to reduce the progression of carotid intimal-medial thickness,[11] and pioglitazone was recently shown to lower the incidence of the combined end point of death, myocardial infarction, and stroke in patients with diabetes.[12]

A major question is whether these antiatherosclerotic effects of TZDs are secondary to favorable lipid effects or to other primary effects of PPAR-γ activation. For example, macrophages express PPAR-γ, and TZD treatment of macrophages upregulates ABCA-1, enhances cholesterol efflux, and reduces the macrophage inflammatory response.[13] Studies with PPAR-γ agonists in mice suggest that reduction in atherosclerosis may be mediated through direct effects on macrophages.[14]

Although there are substantial data on the effects of TZDs in persons with diabetes, the body of data regarding TZDs in persons without diabetes is much less complete. To investigate the potential for direct effects of rosiglitazone on lipids and inflammatory markers, we performed a prospective,

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double-blinded study in which we randomized nondiabetic subjects with low HDL-C and metabolic syndrome to receive either rosiglitazone 8 mg daily or a matching placebo for 12 weeks. Our goal was to test the effect of rosiglitazone on lipids, adipokines, and inflammatory proteins in this subgroup of patients.

Methods

Study Participants

The study was approved by the Institutional Review Committees at the University of Pennsylvania and the Philadelphia Veterans Affairs Medical Center, and all participants provided written informed consent. Subjects were enrolled from outpatient practices between April 2003 and October 2004. The enrolled subjects included men and women between the ages of 18 and 75 years, with low HDL-C (<40 mg/dL for men and <50 mg/dL for women), and at least 2 of the following criteria: (1) abdominal obesity (waist circumference ≥40 inches in men and ≥35 inches in women); (2) blood pressure ≥130/85 mm Hg or the ongoing use of an antihypertensive agent; (3) fasting serum glucose level ≥110 mg/dL; and (4) fasting serum triglyceride level ≥150 mg/dL. Subjects were excluded if they had an LDL-C level ≥190 mg/dL, diabetes (defined by the use of antihyperglycemic medication or a fasting glucose ≥125 mg/dL), uncontrolled hypertension (>180/100 mm Hg), a serum triglyceride level >800 mg/dL, hepatic disease, vascular disease, a chronic inflammatory disorder, surgery within 30 days, active substance abuse, or any lipid-lowering therapy within the preceding 6 weeks.

Study Protocol

Subjects were randomized in a double-blind fashion to either rosiglitazone 8 mg or an identical matching placebo once daily for 12 weeks (GlaxoSmithKline, Inc). Randomization was performed by an unblinded investigational pharmacist, using a random number generator (Rando; Hawkeye Softworks). Participants were instructed to maintain their usual dietary and exercise habits. Study assessments were performed at baseline, 6 weeks, and 12 weeks for the following: (1) weight (Scale-Tronix digital scale; Scale-Tronix) and waist circumference; (2) blood pressure; (3) diet stability (through dietary records, obtained for 3 days before each visit, and analyzed with The Minnesota Nutrition Data System version 4.06 (University of Minnesota, Minneapolis, Minn); (4) exercise frequency (categorized as daily, 2 to 3 times per week, once per week, once per month, or never); (5) alcohol use; (6) bioelectrical impedance assessment of body composition (Quantum II Analyzer, RJL Systems); and (6) fasting blood samples for laboratory analyses.

Laboratory Analyses

Lipoprotein analyses were performed at baseline and 12 weeks on samples obtained after a 12-hour fast in a Centers for Disease Control–standardized lipid laboratory. Levels of total cholesterol, HDL-C, and triglycerides were measured with a Cobas Fara II autoanalyzer (Roche Diagnostic Systems, Inc) using Sigma reagents (Sigma Chemical Co). Very low-density lipoprotein cholesterol (VLDL-C) levels were determined after ultracentrifugation at a density of 1.006 g/mL. Levels of lipoprotein(a) [Lp(a)] were measured using DiaSorin reagents (DiaSorin Inc). Levels of free fatty acids, phospholipids, free cholesterol, apolipoprotein (apo)A-I, apoA-II, apoB, and apoC-III were measured on a Hitachi 912 autoanalyzer using Wako reagents (Wako Pure Chemical Industries). Insulin, leptin, and adiponectin levels were measured by radioimmunoassay (Linco Research, Inc). Lipoprotein subfractions were measured by proton nuclear magnetic resonance spectroscopy (LipoScience, Inc). Insulin resistance was estimated with the homeostasis model assessment or HOMA (plasma insulin [μIU/mL]×plasma glucose [mmol/L]÷22.5). Samples were assayed for CRP with an ultra-high sensitivity latex turbidometric immunoassay (Wako Pure Chemical Industries Ltd) on a Hitachi 912 autoanalyzer. Plasma resistin levels were measured by enzyme immunoassay (Linco Research, Inc).[15] Plasma levels of IL-6 and sTNF-α R^2 (the soluble cleavage product of the activated tumor necrosis factor [TNF]-α receptor) were measured with a commercially available enzyme immunoassays (R&D Systems).

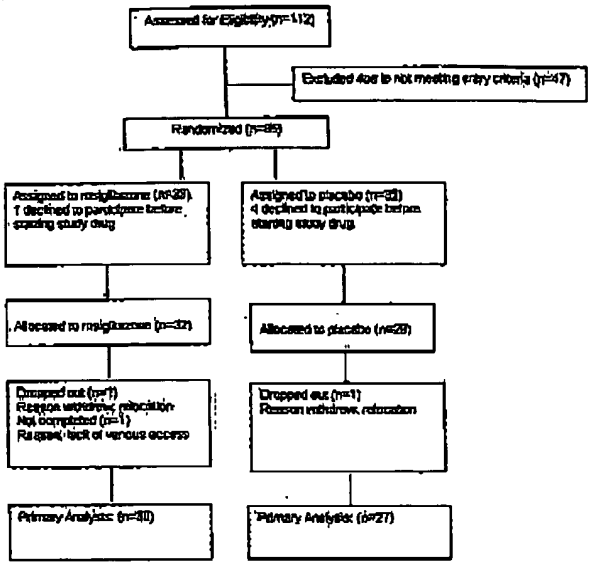

Diagram of the flow of subjects through the study.

Statistical Analysis

We estimated a 10%±14% greater increase in HDL-C with rosiglitazone versus placebo.[4,5,7] To have 80% power to detect this difference, with a 2-tailed α=0.05, we estimated the need for a sample size of 52 subjects. This sample size also provided 90% power, with a 2-tailed α of 0.05, to detect a 25% greater decrease in CRP with rosiglitazone, compared with placebo.[10] Based on an estimated 15% dropout rate, we chose to enroll 60 total subjects. Only subjects who completed the study were included in the primary analyses.

Comparisons between groups for continuous variables were performed with analysis of covariance models, each containing the baseline value of the response variable and an indicator variable for treatment group. All data were assessed for a normal distribution before analyses. Levels of triglycerides, free cholesterol, free fatty acids, apolipoprotein C III, Lp (a), insulin, certain LDL and HDL subfractions, CRP, IL-6, sTNF-α, adiponectin, and insulin resistance (HOMA) were skewed, and thus log-transformed before analyses. Resistin levels were not completely normalized by log-transformation and were thus analyzed using the Mann-Whitney test (Wilcoxon rank sum test). Dichotomous variables were compared by χ^2 analysis or logistic regression analyses. Comparisons of dietary nutrient composition between groups were made using the unpaired t test. Pearson correlation analyses were used to test correlations between non-normally distributed variables. Normally distributed continuous variables are given as means with SD, and non-normally distributed variables are given as medians with inter-quartile ranges. All analyses were performed using SPSS 11.5 (SPSS, Inc).

Results

The final study group included 30 subjects assigned to receive rosiglitazone and 27 subjects assigned to receive placebo (Figure). One subject was missing data for measurements of lipoprotein particle sizes, adipokines, and inflammatory markers, because of a laboratory error.

Fifty-three percent of the participants were black. There were no significant differences in baseline characteristics

TABLE 1. Baseline Characteristics of the Participants

Subject Characteristics	Rosiglitazone (n=30)	Placebo (n=27)	*P*
Age, years	54±8	57±9	0.12
Male sex	26 (87)	24 (89)	0.80
Race			
Black	16 (53)	14 (52)	0.91
White	11 37)	11 (41)	0.75
Hispanic	2 (7)	2 (7)	0.91
Native American	1 (3)	0 (0)	0.34
Weight, kg	108±15	105±18	0.50
Body mass index, kg/m²	35±5	34±6	0.85
Waist circumference, inches	45±4	43±10	0.37
Systolic blood pressure, mm Hg	138±16	134±12	0.33
Diastolic blood pressure, mm Hg	82±12	79±10	0.36
Current smokers	10 (33)	13 (48)	0.26
Current ethanol users	10 (33)	13 (48)	0.26
Concomitant medications			
Aspirin	6 (20)	5 (19)	0.89
Nonsteroidal antiinflammatory medications	11 (37)	10 (37)	0.98
Antihypertensives	14 (47)	17 (63)	0.22

Variables given as means±SD, or as n (% of treatment group).

between the study groups (Table 1). There was no significant difference in exercise frequency between the groups at baseline (P=0.24) or 12 weeks (P=0.70) (data not shown). No subjects started lipid-lowering therapy during the study.

Changes in Weight, Blood Pressure, Insulin Resistance, and Free Fatty Acids

There was no significant effect of rosiglitazone on body weight (P=0.51), waist circumference (P=0.15), dietary intake (all P>0.41), or percent fat mass (P=0.085) (data not shown). Systolic blood pressure decreased by 9±15 mm Hg on rosiglitazone, but also decreased by 5±13 mm Hg in the placebo group (P=0.68). Diastolic blood pressure decreased by 2±10 mm Hg on rosiglitazone group and increased by 3±11 mm Hg on placebo (P=0.16).

The decrease in insulin resistance (as estimated by the HOMA equation) (-26% in both groups; P=0.23) and in free fatty acid levels (−16% versus −4%, respectively; P=0.16) (Table 2) were not significantly different between the rosiglitazone and placebo groups.

Changes in High-Density Lipoproteins

There was no significant difference in the change in HDL-C level between the rosiglitazone and placebo groups (+5.5% versus +5.8%, respectively; P=0.89) or apolipoprotein A-I levels (−1.4% versus +0.6%; P=0.10), but a significantly greater increase in apolipoprotein A-II (+16% versus +3%; P<0.001) in the participants receiving rosiglitazone, compared with placebo (Table 3). The subjects receiving rosiglitazone experienced a greater decrease in large HDL (P=0.038) particles and a greater increase in medium-sized HDL particles (P=0.033). There was no significant change in small HDL particle concentration (P=0.092) (Table 4).

Changes in ApoB-Containing Lipoproteins

As shown in Table 3, there were greater increases in levels of total cholesterol (+9% versus −2%; P=0.030), free (unesterified) cholesterol (+14% versus −2%; P=0.015), and non-HDL-C (+9% versus −2%; P=0.028) in the group receiving rosiglitazone compared with placebo. ApoB also increased more on rosiglitazone, (+10% versus −2%; P=0.021). Neither LDL-C (+0% versus −4%; P=0.42), VLDL-C (+40% versus +2%; P=0.076) or triglyceride levels (+4% versus −3%; P=0.40) responded significantly differently in those receiving rosiglitazone compared with placebo. We did not find any significant effects of rosiglitazone treatment on LDL particle size (Table 4).

TABLE 2. The Response of Insulin, Glucose, and HOMA-IR to Treatment

Variable (n=29 and 27)	Baseline	12 Weeks	Change	*P*
*Insulin, μU/mL				0.76
Rosiglitazone	18.6 (15.7 to 31.1)	17.0 (11.2 to 28.7)	−4.6 (−8.6 to 1.7)	
Placebo	17.2 (11.2 to 23.3)	16.3 (11.0 to 21.6)	−3.5 (−5.7 to 3.0)	
Glucose, mg/dL				0.77
Rosiglitazone	83±16	80±13	−3±12	
Placebo	90±10	82±17	−8±17	
*HOMA-IR				0.23
Rosiglitazone	4.3 (2.4 to 6.6)	3.6 (2.1 to 5.6)	−1.1 (−2.3 to 0.5)	
Placebo	3.8 (2.4 to 5.4)	3.2 (2.1 to 4.5)	−1.0 (−1.7 to 0.3)	
*Free fatty acids, mEq/L				0.16
Rosiglitazone	0.43 (0.34 to 0.63)	0.38 (0.25 to 0.52)	−0.07 (−0.21 to 0.03)	
Placebo	0.56 (0.37 to 0.67)	0.52 (0.35 to 0.70)	−0.02 (−0.17 to 0.08)	

Values with an asterisk are skewed and given as the median with interquartile range. Other values are given as means±SD. *P* are for between-group comparisons of the changes between baseline and 12 weeks. Two subjects were excluded from the analysis of insulin, glucose, and HOMA-IR because of outlier values for fasting insulin (>100 μU/mL).

TABLE 3. The Response of Lipid and Lipoprotein Levels to Treatment

Variable (n=30 and 27)	Baseline	12 Weeks	Change	P
Total cholesterol, mg/dL				0.030
Rosiglitazone	202±30	219±44*	16±40	
Placebo	197±36	195±37	−2±23	
VLDL-C, mg/dL				0.076
Rosiglitazone	38±20	53±56	15±44	
Placebo	41±19	42±29	1±21	
LDL-C, mg/dL				0.42
Rosiglitazone	129±29	129±36	0±31	
Placebo	121±35	117±38	−5±24	
HDL-C, mg/dL				0.89
Rosiglitazone	38±5	37±7	2±6	
Placebo	34±5	36±6	2±5	
Non-HDL-C, mg/dL				0.028
Rosiglitazone	167±29	181±44	15±40	
Placebo	162±37	159±36	−3±22	
*Triglycerides, mg/dL				0.40
Rosiglitazone	158 (119 to 293)	182 (105 to 236)	6 (−33 to 54)	
Placebo	190 (137 to 262)	160 (116 to 272)	−6 (−60 to 34)	
*Lp(a), mg/dL				0.55
Rosiglitazone	20.0 (11.2 to 42.6)	16.6 (10.8 to 35.7)	−0.5 (−4.5 to 1.8)	
Placebo	22.7 (7.7 to 56.1)	18.8 (5.5 to 46.1)	1.7 (−3.9 to 3.6)	
*Free cholesterol, mg/dL				0.015
Rosiglitazone	50 (45 to 53)	53 (51 to 61)	7 (2 to 11)	
Placebo	50 (44 to 57)	52 (44 to 59)	−1 (−3 to 3)	
ApoA-I, mg/dL				0.10
Rosiglitazone	99.9±22.6	97.6±13.4	−1.4±21.1	
Placebo	102.9±8.5	103.9±9.1	0.7±10.4	
ApoA-II, mg/dL				<0.001
Rosiglitazone	28.3±3.7	32.7±4.2	4.4±2.5	
Placebo	28.9±2.5	29.6±2.6	0.8±1.5	
ApoB, mg/dL				0.021
Rosiglitazone	92±21	100±18	9.4±25.2	
Placebo	94±16	92±18	−2.8±9.4	
*ApoC-III, mg/dL				0.15
Rosiglitazone	10.1 (7.5 to 12.1)	10.8 (8.7 to 14.5)	1.1 (−0.9 to 2.6)	
Placebo	10.8 (8.1 to 15.0)	10.4 (8.4 to 15.9)	−0.1 (−2.6 to 2.3)	

*Values are medians with interquartile range. Other values are given as means±SD. P are for between-group comparisons of the changes in variables between baseline and 12 weeks.

Effects on Adipocytokines and Inflammatory Markers

We observed a highly significant 168% increase in adiponectin in the rosiglitazone group, compared with a 25% increase in the placebo group ($P<0.001$) (Table 5). We also observed a 6% decrease in resistin in the rosiglitazone group, compared with a 4% increase in the placebo group ($P=0.01$) (Table 5). Those assigned to rosiglitazone experienced a greater decrease in CRP level (−32% versus +36%; $P=0.002$), IL-6 (−22% versus +4%; $P=0.027$), and sTNF-α R^2 (−5% versus +7%; $P<0.001$) (Table 5). In the group receiving rosiglitazone, there were significant correlations between the changes in resistin and changes in both IL-6 ($r=0.55$, $P=0.003$) and sTNF-α R^2 ($r=0.40$, $P=0.033$), but not with the change in CRP ($r=0.22$, $P=0.26$). There were no significant correlations between changes in HOMA-IR and any of the inflammatory markers (all $r<0.09$), resistin ($r=-0.26$, $P=0.18$) or adiponectin ($r=0.05$) in the rosiglitazone group

Safety and Tolerability

The average adherence with study medication was 95%±5% for rosiglitazone and 95%±5% for placebo ($P=0.66$). There were 30 mild to moderate adverse events reported by 17 individuals receiving rosiglitazone and 30 mild to moderate events by 14 individuals receiving placebo ($P=0.35$). One subject assigned to rosiglitazone experienced a substantial

TABLE 4. Response of lipoprotein particle distribution by NMR spectroscopy

Variable (N= 29 AND 27)	Baseline	12 Weeks	Change	*P*
LDL particle concentration (umol/L)				0.36
Rosiglitazone	1504±307	1559±363	55±274	
Placebo	1453±389	1446±469	−6±234	
LDL particle size (nm)				0.29
Rosiglitazone	19.8±0.5	20.0±0.7	0.21±0.56	
Placebo	19.9±0.4	19.9±0.6	0.06±0.42	
Small LDL, mg/dL				0.98
Rosiglitazone	1306±335	1289±436	−17±354	
Placebo	1259±398	1241±494	−17±248	
Medium LDL, mg/dL				0.88
Rosiglitazone	259±71	253±91	−6±88	
Placebo	254±68	252±84	−2±53	
*Large LDL, mg/dL				0.108
Rosiglitazone	101 (49 to 209)	141 (80 to 332)	52 (−38 to 141)	
Placebo	120 (62 to 231)	99 (37 to 261)	−8 (−42 to 42)	
HDL particle concentration (μmol/L)				0.46
Rosiglitazone	26±3	26±4	−0.1±4.2	
Placebo	27±3	27±3	−0.1±2.9	
HDL particle size (nm)				0.34
Rosiglitazone	28.0±8.5	28.0±8.4	−0.03±0.25	
Placebo	27.0±8.5	27.0±8.5	−0.01±0.23	
Small HDL, mg/dL				0.092
Rosiglitazone	20.6±3.4	19.0±5.5	−1.6±5.0	
Placebo	22.4±3.7	22.1±3.7	−0.2±3.3	
*Medium HDL, mg/dL				0.033
Rosiglitazone	1.7 (0.4 to 3.8)	3.5 (1.4 to 7.6)	1.8 (−0.2 to 4.3)	
Placebo	1.5 (0.2 to 3.3)	1.7 (0.8 to 3.4)	0.5 (−1.4 to 1.9)	
*Large HDL, mg/dL				0.038
Rosiglitazone	2.2 (1.8 to 3.8)	1.9 (1.0 to 3.0)	−0.6 (−1.3 to 0.2)	
Placebo	2.1 (1.5 to 3.6)	2.1 (1.3 to 3.5)	0.1 (−0.5 to 0.8)	

*Values are given as medians with interquartile range. *P* are for between-group comparisons of the changes in variables between baseline and 12 weeks.

increase in total cholesterol and triglycerides, requiring termination from the study 1 week early.

Discussion

We investigated the effects of rosiglitazone on lipoproteins, adipokines, and inflammatory markers in patients with low HDL-C and metabolic syndrome. Our subjects were obese, highly insulin-resistant, and included 53% blacks. Rosiglitazone modestly increased total cholesterol, LDL-C, and non–HDL-C, had no significant effect on HDL-C, but had favorable effects on adipokines and inflammatory markers.

Our finding that rosiglitazone did not raise HDL-C in patients with metabolic syndrome are consistent with the findings from 3 previous studies of patients without diabetes, 2 involving patients with coronary artery disease[11,16] and 1 involving nonobese patients with metabolic syndrome.[17] We found no effect of rosiglitazone on apoA-1 levels, but an increase in apoA-II levels. ApoA-II has been associated with visceral fat accumulation and impaired catabolism of large VLDL particles.[18] It is thus intriguing to speculate that upregulation of apoA-II may contribute to an increase in VLDL-C levels, the most readily available clinical measure of triglyceride-rich remnant lipoproteins. In our study, mean VLDL-C concentration increased by 40% on rosiglitazone, although this change did not differ significantly from the placebo group.

In contrast, we found overall favorable effects of rosiglitazone on adipokines, including a significant increase in adiponectin levels. This increase in adiponectin may be attributable to a direct effect of rosiglitazone on adipocytes, and possibly macrophages.[19] Adiponectin has been shown to play a role in modulating insulin sensitivity[20] and to be increased by rosiglitazone in patients with diabetes.[20] Furthermore, increasing quintiles of adiponectin levels have been associated with decreased risk of myocardial infarction.[21]

Our study demonstrates that rosiglitazone lowers resistin levels in patients with metabolic syndrome, as recently demonstrated in one small study of 14 patients with type 2 diabetes.[22] Resistin was originally found to be secreted by

TABLE 5. The Response of Adipokines and Inflammatory Markers to Treatment (n=54)

Variable	Baseline	12 Weeks	Change	P
Adiponectin, mg/L				<0.001
Rosiglitazone	6.9 (3.7 to 9.6)	18.7 (10.5 to 28.7)	11.6 (6.4 to 16.0)	
Placebo	7.1 (4.1 to 9.4)	6.9 (5.5 to 12.6)	1.8 (0.7 to 3.8)	
Resistin, ng/mL				0.009
Rosiglitazone	13.2 (11.3 to 17.1)	12.4 (10.5 to 17.7)	−0.8 (−2.0 to 0.2)	
Placebo	13.2 (10.7 to 18.7)	13.8 (10.8 to 20.3)	0.5 (−0.5 to 1.1)	
hs-CRP, mg/L				0.002
Rosiglitazone	1.9 (1.1 to 3.6)	1.7 (0.7 to 2.8)	−0.6 (−1.4 to 0.3)	
Placebo	3.1 (2.1 to 5.4)	3.8 (2.7 to 7.7)	1.1 (−1.1 to 2.7)	
IL-6, pg/mL				0.027
Rosiglitazone	1.8 (1.3 to 2.4)	1.5 (1.0 to 2.2)	−0.4 (−0.9 to 0.5)	
Placebo	2.1 (1.4 to 2.8)	2.2 (1.6 to 2.6)	0.2 (−0.6 to 0.7)	
sTNF-α R2, pg/mL				<0.001
Rosiglitazone	2393 (2043 to 2824)	2194 (1987 to 2725)	−143 (−341 to 36)	
Placebo	2395 (2081 to 2873)	2592 (2071 to 3009)	146 (34 to 280)	

Values are provided as median with interquartile range. *P* are for between-group comparisons of the changes in variables between baseline and 12 weeks. Two additional subjects (1 from each group) were excluded in the analyses for inflammatory markers and adipokines, one because of a urinary tract infection, and another because of gout.

adipocytes in mice, in which it caused impaired insulin action.[23] In humans, however, resistin is predominantly produced by macrophages in response to inflammatory stimuli and is almost undetectable in adipose tissue.[15] Rosiglitazone may thus have a direct effect on resistin expression, such as through macrophage PPAR-γ activation. We have recently shown that resistin levels independently correlate with degree of coronary artery calcification.[15]

Rosiglitazone also significantly reduced levels of CRP, IL-6, and sTNF-α R^2. Similar responses for CRP and IL-6 were previously found in patients with diabetes.[10] We found a significant correlation between the rosiglitazone-induced changes in resistin and changes in the inflammatory markers IL-6 and sTNF-α R^2. These findings are consistent with our previous findings of a significant correlation between baseline levels of resistin and sTNF-α R^2.[24] Rosiglitazone has been shown to lower CRP in nondiabetic patients with coronary artery disease[16] and in nonobese Taiwanese patients with metabolic syndrome.[17]

The HDL effects of rosiglitazone differ from those of pioglitazone. The recently published GLAI study directly comparing rosiglitazone and pioglitazone in patients with diabetes showed a 14.9% increase in HDL-C with pioglitazone versus a 7.8% increase with rosiglitazone.[25] Furthermore, we recently performed a study with pioglitazone in patients with metabolic syndrome that was similar in design to the current study, and showed a 14% increase in HDL-C with pioglitazone.[26] In patients with diabetes, pioglitazone also reduced triglycerides to a greater extent than rosiglitazone,[25] although this could have been influenced by their selection of patients with elevated baseline triglyceride levels. Similarly, in our 2 separate studies in nondiabetic subjects with metabolic syndrome, pioglitazone reduced triglycerides more than rosiglitazone. The mechanism underlying these differential effects between agents considered to be ligands for the same PPAR-γ receptor remains unclear. However, a previous study has shown incompletely overlapping transcriptional regulation by TZDs.[27] Pioglitazone has been suggested to have modest effects on activating PPAR-α, which could contribute to its more potent effects on HDL-C and triglyceride levels.[28] Whereas neither apoA-I production rate nor fractional catabolic rate appears to be affected by pioglitazone, apoC-III was reduced by pioglitazone, consistent with a PPAR-α effect.[29] This effect may account for the decrease in triglyceride concentration with pioglitazone, and could indirectly increase HDL-C via CETP-mediated exchange of VLDL triglycerides for HDL cholesterol. Interestingly, pioglitazone has been shown to increase expression of fatty acid oxidation enzymes in adipose tissue, as well as increase expression of PPAR-α itself, which might also contribute to changes in triglycerides and/or HDL.[30] Both rosiglitazone and pioglitazone exerted potent antiinflammatory effects and more than doubled adiponectin levels in our studies.

Although randomized, double-blinded, and placebo-controlled, our study had important limitations. We estimated insulin resistance through the use of the HOMA index, whereas dynamic measures, such as the frequently sampled intravenous glucose tolerance test, are better validated. Our observed 26% reduction in HOMA-IR with rosiglitazone is consistent with that found in previous studies in patients without diabetes.[16,17] The fasting glucose levels in the control group were higher at baseline than in the rosiglitazone group, and the comparable decrease in HOMA-IR in the control group may be attributable to the decrease in fasting glucose level in this group between baseline and follow-up, representing regression to the mean. There was no measurable change in weight, exercise, or other lifestyle that would otherwise have accounted for this change. Third, we used a 12-week treatment period, and 1 previous study suggested that the

metabolic response to rosiglitazone may evolve over a 1-year period of time.[9] Last, our study was also limited by the inclusion of a small number of women.

In this prospective, double-blinded study, in which 60 nondiabetic patients with metabolic syndrome were randomized to rosiglitazone 8 mg or placebo for 12 weeks, we found that rosiglitazone had modestly unfavorable overall effects on plasma lipids but significantly raised adiponectin levels and reduced resistin as well as other markers of inflammation. Thus, our data support the concept that the potential antiatherosclerotic effects of rosiglitazone may be partly related to direct effects on adipokines and inflammation. The ongoing Rosiglitazone Evaluated for Cardiac Outcomes and Regulation of Glycemia in Diabetes (RECORD) Trial will test whether rosiglitazone lowers the incidence of cardiovascular events. A reduction in cardiovascular events with rosiglitazone in this trial would more likely reflect rosiglitazone's favorable effects on adipokines and inflammation than its lipid-related effects.

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EXHIBIT D

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Oral Therapy in Type 2 Diabetes: Pharmacological Properties and Clinical Use of Currently Available Agents

Charles M. Clark Jr., MD

Abstract

Type 2 diabetes is associated with not only hyperglycemia, but also other atherogenic risk factors, including hypertension and dyslipidemia. Thus, the objectives in the management of type 2 diabetes are to maintain blood glucose levels within an acceptable range and to treat co-existing conditions, thereby diminishing the incidence and severity of long-term complications. The mainstays of type 2 diabetes management are dietary and lifestyle modifications. However, when these measures fail to maintain adequate glycemic control, oral drug therapy is initiated. Available pharmacological agents include the sulfonylureas, the biguanide metformin, acarbose, troglitazone, and repaglinide. Pharmacological differences among these agents may determine their appropriateness for specific patient groups. Although all of these agents are effective in treating type 2 diabetes, metformin and troglitazone may offer additional benefits with regard to the obesity and dyslipidemia associated with the disease.

Type 2 diabetes (non-insulin-dependent diabetes mellitus) is a metabolic disorder whose etiology and pathogenesis are not completely understood,[1] yet it constitutes 92% of cases of diabetes in the United States. The two primary pathogenic factors leading to type 2 diabetes are insulin resistance and decreased insulin secretion, which arise from abnormalities within the liver, skeletal muscle, and pancreatic β-cells.

The extent of these defects varies among type 2 diabetic patients. Impaired insulin sensitivity occurs at the tissue (liver and skeletal muscle) level, and it is a predominant finding in overweight diabetic patients. Impaired insulin secretion occurs at the level of the pancreatic β-cell and occurs primarily in lean diabetic individuals.[2] Although large-scale studies have yet to confirm which of these abnormalities prevails, recent studies in small patient populations suggest that insulin resistance may be the primary defect,[3,4] but that a defect in insulin secretion is a prerequisite for progression to type 2 diabetes.[2]

Insulin resistance is characterized by decreased uptake and metabolism of glucose in skeletal

muscle[3-5] and by hepatic overproduction of glucose. Patients with insulin resistance have significant hyperglycemia, even though their plasma insulin levels may be normal or increased.

The hyperinsulinemia that occurs in these patients is compensatory, as the pancreatic β-cells attempt to reduce hyperglycemia.[3,5] Hyperinsulinemia also reflects impaired insulin sensitivity and may result from defects at the level of the insulin receptor, in messenger signals, or in the glucose transporter system.[3,4]

Moreover, insulin resistance is associated with several atherogenic abnormalities that increase the risk of coronary heart disease (CHD).[6-10] These include increased plasma triglyceride levels, decreased high-density lipoprotein (HDL) cholesterol levels, and hypertension. The combination of insulin resistance and its associated atherogenic risk factors has been referred to as "Syndrome X."[6-11]

The second pathogenic factor, defective insulin secretion, is believed to contribute to the development of overt type 2 diabetes, particularly fasting hyperglycemia. Although fasting plasma insulin is frequently increased in patients with type 2 diabetes, insulin secretory defects in the pancreatic β-cell are also present. These defects appear to occur early in the course of the disease; in the majority of individuals there is a loss of first-phase insulin response to an intravenous glucose challenge. Therefore, defects in both insulin resistance and insulin secretion contribute to the etiology of type 2 diabetes.[3,4]

As the disease progresses and hyperglycemia worsens, pancreatic β-cells no longer adequately respond to the glycemic stimulus, and insulin secretion declines. This combination of effects results in chronic hyperglycemia, which further impairs insulin secretion and action.[2,3,12,13] This concept of chronic increments in plasma glucose levels leading to progressive impairment of insulin secretion is referred to as "glucose toxicity" and is an important aspect of type 2 diabetes treatment.[2,3]

Table 1. Targets for Metabolic Control and Body Mass Index in Type 2 Diabetes Patients[16]

Target	Ideal	Acceptable
Fasting plasma glucose (mmol/L)	4.4–6.7	<7.8
Postprandial plasma glucose (mmol/L)	4.4–8.9	<10
Glycosylated hemoglobin* (%)	<mean + 2SD	<mean + 4SD
Total cholesterol (mmol/L)	<5.2	<6.5
HDL cholesterol (mmol/L)	>1.1	>0.9
Fasting triglycerides (mmol/L)	<1.7	<2.2
Body mass index		
Men (kg/m^2)	<25	<27
Women (kg/m^2)	<24	<26

*Mean and standard deviation (SD) for nondiabetic individuals determined by each particular hospital laboratory. These values correspond to 1% and 2% above the normal range. For HbA_{1c} with an upper limit of normal of 6%, for example, the ideal would be <7% and acceptable <8%.

Because type 2 diabetes is frequently associated with hyperglycemia-induced long-term complications, hypertension, obesity, and lipid abnormalities, early detection and management of type 2 diabetes is important. The primary treatment objective in the management of type 2 diabetes is to achieve and maintain good glycemic control. Controlling co-existing conditions, detecting and treating chronic complications (e.g., retinopathy and neuropathy), and avoiding hypoglycemia are secondary objectives.[8,14,15] Table 1 summarizes targets for metabolic control and body mass index in type 2 diabetic patients.[16] In overweight individuals, these abnormalities improve with weight reduction and exercise. Only when this approach has been ineffective should drug therapy be initiated.[17]

Table 2. Pharmacological Differences Among Metformin, Sulfonylureas, Acarbose, Troglitazone, and Repaglinide[18-23]

Characteristic	Metformin	Sulfonylureas	Acarbose	Troglitazone	Repaglinide
Mechanism of action	↓ hepatic glucose production and gluconeogenesis ↑ peripheral glucose utilization ↓ intestinal glucose	↑ pancreatic insulin secretion	↓ digestion of complex carbohydrates and disaccharides to absorbable monosaccharides	↓ hepatic glucose production and gluconeogenesis ↑ peripheral glucose utilization	↑ pancreatic insulin secretion

	absorption				
Blood glucose levels	↓ in hyperglycemic state only	↓ in hyperclycemic and normoglycemic states	↓ in hyperclycemic state only	↓ in hyperclycemic state only	↓ in hyperclycemic and normoglycemic states
Plasma insulin levels	↓ or unchanged	↑	Unchanged	↓	↑
Body weight	↓ or unchanged	↑	↓ or unchanged	Unchanged	Not known
Plasma lipids in type 2 diabetes patients	↓ LDL cholesterol, total cholesterol, triglycerides ↑ or no effects on HDL cholesterol	No significant effects	No consistent effects	↑ LDL cholesterol, total cholesterol, ↓ triglycerides ↑ or no effects on HDL cholesterol	No significant effects

↑ = Increased/enhanced; ↓ = Decreased/suppressed

PHARMACOLOGY OF ANTIDIABETIC AGENTS

Antidiabetic agents available for the treatment of type 2 diabetes include the sulfonylureas, the biguanide metformin, the α-glucosidase inhibitor acarbose, the thiazolidinedione troglitazone, and the meglitinide repaglinide. Although these therapies are effective antidiabetic agents, their mechanisms of action differ (Table 2).[18-23]

Sulfonylureas

Sulfonylureas work predominantly through pancreatic mechanisms, although extrapancreatic mechanisms may also contribute to their antihyperglycemic effects.[14,24]

Pancreatic Effects

Sulfonylureas stimulate the release of insulin from pancreatic β-cells. Studies in which sulfonylureas have been administered to pancreatectomized animals and to patients with type 1 (insulin-dependent) diabetes mellitus and pancreatic diabetes have demonstrated that these agents exhibit a hypoglycemic effect only if a functional pancreas is present.[19]

The mechanism by which sulfonylureas stimulate insulin secretion appears to be receptor-mediated. Insulin secretion is initiated when the drug binds to a cell surface receptor on the pancreatic β-cell.[14,19] This interaction inhibits the efflux of potassium ions and causes depolarization. This depolarization then causes a calcium channel to open, which in turn causes an influx of calcium, leading to the release of insulin.

Extrapancreatic Effects

Although it has been proposed that numerous extrapancreatic effects may contribute to the hypoglycemic effect of sulfonylureas, it is unlikely that these effects are clinically relevant. Two mechanisms in particular have been reported[19,25] involving the potentiation of insulin action—on the liver to decrease hepatic glucose production and on skeletal muscle and adipose tissue to improve carbohydrate transport. However, the relevance of these actions to the overall efficacy of the sulfonylureas is modest.

Metformin

Unlike the sulfonylureas, which are classified as hypoglycemic agents, metformin is more accurately described as an antihyperglycemic agent because it does not cause hypoglycemia when used alone in type 2 diabetic patients. Metformin lowers plasma glucose levels in type 2 diabetes by reducing insulin resistance. This pharmacodynamic action partially reverses the underlying pathophysiology of Syndrome X. Metformin monotherapy-induced reduction of hyperglycemia is similar to that achieved with sulfonylureas in obese and nonobese patients.[22,26-29]

Hepatic Glucose Production (HGP)

As a result of insulin resistance, excessive HGP is a major feature of type 2 diabetes and a major contributor to the hyperglycemic condition.[3,30] Metformin reduces HGP by 9–30% in patients receiving metformin, relative to baseline or placebo, usually after administration of metformin dosages ranging from 1,000 to 2,550 mg/day for up to 3 months.[31-36] This decrease in HGP is attributed to an inhibition of gluconeogenesis.[14,18,36]

Peripheral Glucose Utilization

Peripheral insulin-mediated glucose utilization is 20–40% lower in diabetic individuals compared with nondiabetic controls.[29,30] The administration of metformin 0.5–3 g/day for up to 3 months increased peripheral glucose utilization by 18–29% relative to baseline or placebo.[33,37-41] This improvement in glucose utilization occurs in skeletal muscle, in fat and intestinal tissue, and possibly in erythrocytes.[18,30]

Other Antihyperglycemic Effects

Although metformin has been shown to decrease intestinal glucose absorption, this does not sufficiently account for the significant antihyperglycemic effect of metformin.[42]

Evidence from studies conducted in animals and cultures of skeletal muscle taken from insulin-resistant patients indicates that metformin acts at the cellular level to enhance glucose transport by stimulating glucose transporter activity.[38,43,44] Again, the clinical significance of this observation is unknown.

Acarbose

Acarbose is the first drug in a new class of antidiabetic agents, the α-glucosidase inhibitors, that

has recently become available in the United States for the treatment of type 2 diabetes. It exerts its antidiabetic effect by delaying the digestion of complex carbohydrates and disaccharides (e.g., starch) to absorbable monosaccharides (e.g., glucose). This is accomplished by reversible inhibition of the α-glucosidase enzymes (e.g., sucrase and maltase) that are located in the duodenum.[16,20] In type 2 diabetic patients, this enzyme inhibition delays glucose absorption following ingestion of complex carbohydrates.

Acarbose does not appear to have a direct effect on insulin resistance or on insulin-stimulated glucose uptake in humans.[20]

Troglitazone

Troglitazone is the first drug in another new class of oral antidiabetic agents, the thiazolidinediones. Thiazolidinediones are thought to produce their antidiabetic effect by activating the peroxisome proliferator-activated receptor (PPAR)g, a nuclear receptor that regulates the transcription of several key insulin-sensitive genes involved in controlling glucose and lipid metabolism.[21] Like metformin, troglitazone alleviates hyperglycemia in type 2 diabetic patients by reducing insulin resistance in peripheral tissues.

Troglitazone monotherapy at dosages of 400 and 600 mg/day increases insulin-mediated glucose disposal (by ~45%) after 6 months of treatment.[45] A reduction in HGP in type 2 diabetic patients occurred only after administration of the maximum recommended dose of troglitazone (600 mg/day) for 6 months in one study ($n = 93$)[45] and after administration of troglitazone (200 mg twice daily) for 6–12 weeks in a much smaller study ($n = 11$).[46] The reduction in HGP appears to be secondary to suppression of gluconeogenesis.[21]

The antihyperglycemic effect of troglitazone appears to be intermediate between that of acarbose and that of metformin and the sulfonylureas. Troglitazone monotherapy at doses of 200, 400, and 600 mg/day for 3 months reduced fasting serum glucose by 11, 14, and 15%, respectively, in a dose-response study.[47] Little additional benefit was gained by increasing the dose to 800 mg/day, at which a 19% reduction in fasting serum glucose was observed. Results of two separate studies in type 2 diabetic patients suggest that troglitazone 400 mg/day achieves equivalent reductions in hyperglycemia as glyburide 12 mg/day after 1 year of treatment[48] and as metformin 2,000 mg/day after 3 months of treatment.[49] However, these data need to be confirmed in other trials, particularly the data relating to metformin, as the latter study involved only 28 patients.

Repaglinide

Repaglinide is another first drug of its class, the meglitinides, which are benzoic acid derivatives. The Food and Drug Administration approved it in April 1998 for use alone and in combination with metformin. Repaglinide is an insulin secretagogue with a rapid onset of action and a short half-life.

The few clinical trials have been summarized in The Medical Letter.[23,50] When compared to the sulfonylurea glyburide, repaglinide was less effective in lowering fasting plasma glucose but more effective in lowering postprandial glucose. Similar effects on hemoglobin A_{1c} were seen. A lowering of HbA_{1c} of between 1.3 and 1.9% can be expected, similar to the sulfonylureas.

Repaglinide works directly on the pancreatic β-cell and thus is ineffective in type 1 diabetes.

When used in combination with metformin, repaglinide is synergistic, as would be expected given the two drugs' different mechanisms of action.[51]

Side effects in the clinical trials were not significantly different between the drug and placebo, with the exception of hypoglycemia, which in our limited experience appears to occur less frequently than with the sulfonylureas. Repaglinide needs to be taken with meals, with dosing dependent on initial HbA_{1c} and clinical response. Initial doses range between 0.5 and 2 mg, with total daily dose not to exceed 16 mg.

Secondary Pharmacological Effects Effect on Insulin Levels

In contrast to sulfonylureas, neither metformin nor troglitazone causes hyperinsulinemia.[18,21,29,52] A reduction in insulin levels has been observed when metformin was administered alone or in combination with a sulfonylurea.[27,53] In a 3-year study comparing diet, insulin, metformin, and sulfonylureas, mean fasting plasma insulin levels significantly increased ($P<0.001$) in all but the metformin treatment group.[29] In the same trial, significant reductions (-1.6 μU/L; $P<0.001$) in fasting plasma insulin levels were found in overweight patients receiving metformin, compared with overweight patients utilizing a change in diet alone.

Troglitazone generally reduces fasting [45,47,54,55] and postprandial[46]plasma insulin levels. Reductions from baseline in plasma insulin levels of 5–31% were reported after 3 months of treatment with troglitazone dosages ranging from 200 to 800 mg/day.[47,54] However, in one placebo-controlled 6-month monotherapy study, only the 600 mg/day dose (the maximum recommended in the United States) produced significant reductions ($P < 0.01$) in plasma insulin compared with placebo.[55] In combination with a sulfonylurea (glyburide), troglitazone 600 mg/day produced significant reductions in insulin levels compared with placebo after 6 weeks' treatment.[56]

Clinical trials are not entirely consistent with regard to the effect of acarbose on plasma insulin. Placebo-controlled and noncomparative investigations have reported changes in fasting or postprandial insulin levels, but these changes were not statistically significant.[20,57] Of three randomized double-blind studies, two reported substantial decreases in postprandial insulin levels compared with placebo.[58-60]

Effect on Lipids

Dyslipidemia, including decreased plasma HDL cholesterol levels, is usually present in those with poorly controlled type 2 diabetes. As these abnormalities are now recognized to be major contributors to the development of arterial vascular disease, there is significant interest in the beneficial effects of antidiabetic agents, particularly those that reduce insulin resistance, such as metformin[18] and troglitazone,[21] on lipid metabolism.

Improvements in glycemic control during sulfonylurea therapy have been associated with decreases in plasma total cholesterol, total triglyceride, very-low-density lipoprotein (VLDL) cholesterol and low-density lipoprotein (LDL) cholesterol levels, and either an increase or no change in HDL cholesterol levels.[14,61,62] However, some studies have demonstrated small increases[22] or only small decreases[63,64] in plasma total and LDL cholesterol and triglycerides.

Metformin therapy has generally produced reductions in plasma triglyceride and total and LDL cholesterol, with little or no effect on HDL cholesterol in type 2 diabetic patients.[65-68] The

favorable effect of metformin on plasma total cholesterol levels is reported to be significantly greater

($P < 0.05$) than that of glyburide.[63,64] Reductions in plasma total triglyceride and total cholesterol levels appear to be a result of decreased LDL or VLDL cholesterol.[65]

It has been suggested that the effect of metformin on lipids is independent of its antihyperglycemic effect.[18,68] Although the exact mechanism by which these lipid changes occur has not been determined, they may occur as a result of a direct effect of metformin on VLDL cholesterol metabolism and/or secondarily to improved insulin sensitivity.[68]

Acarbose also reduces serum triglyceride concentrations but has little or no effect on total serum cholesterol concentrations. This effect appears to be mediated by suppressing the biosynthesis of VLDL cholesterol.[57] A review of more recent studies reported that fasting triglyceride levels were reduced, but only occasionally.[20] This effect appears to be associated with dosages >100 mg three times a day.[59,60,69,70] Of the studies that used dosages <300 mg/day, none demonstrated statistically significant changes, relative to baseline or placebo, in fasting triglyceride, total cholesterol, or cholesterol fractions.[60]

Troglitazone induces increases from baseline in both LDL cholesterol (by 5–15%) and total cholesterol (by 1–8%).[54,55] In two placebo-controlled studies, the increase in LDL cholesterol was significant ($P < 0.05$), compared with the change seen with placebo.[54,55] However, the cholesterol/HDL and LDL/ApoB ratios were unchanged, suggesting no change in atherogenic risk. In these studies, only the 600 mg/day dosage resulted in a significant increase ($P < 0.01$ vs. placebo) in HDL cholesterol (by ~10–12%). However, 6- to 12-week studies failed to observe increases in HDL cholesterol during treatment with troglitazone 200–800 mg/day either alone[71,72] or in combination with a sulfonylurea.[72,73]

Troglitazone reduces serum triglyceride levels, but in two monotherapy studies, only the 600 mg/day dosage produced significant reductions ($P < 0.05$) compared with placebo.[45,54] Serum triglycerides decreased from baseline by ~19% at the 600 mg/day dosage ($P < 0.05$ vs. placebo), by ~11% at the 200 and 400 mg/day dosages during monotherapy,[54] and by ~12% ($P < 0.001$ vs. baseline) during combination therapy with troglitazone 400 mg/day and a sulfonylurea.[73] At a troglitazone dosage of 800 mg/day, which is higher than the recommended maximum dose, significant ($P < 0.05$ vs. placebo or vs. baseline) reductions in serum triglycerides (by 14% and 32%) and significant ($P < 0.05$) increases in HDL cholesterol (by 16% in one study) have been observed.[54,74]

Effect on Body Weight

In comparative studies of sulfonylureas and metformin, sulfonylurea therapy resulted in mean weight gains ranging from 2.6 to 5.3 kg, whereas metformin treatment was associated with either no change or a modest reduction in body weight.[22,26,28,29,75] The stabilization or reduction in body weight noted with metformin therapy may be clinically beneficial, since the majority of type 2 diabetic patients are overweight.

Comparative and noncomparative studies have reported inconclusive findings regarding the effects of acarbose on body weight, with the majority of investigations failing to demonstrate that acarbose

has an effect on body weight in either lean or obese patients.[57] In a recent trial comparing acarbose, metformin, and insulin therapy as adjunctive therapy to sulfonylurea treatment failures, body weight increased in the insulin group and decreased in both the metformin and acarbose groups. The reduction in body weight was 1.2 ± 1.9% in the metformin group and 0.6 ± 1.6% in the acarbose group. This difference was not statistically significant.[76]

In clinical trials of troglitazone monotherapy, mean body weight was unchanged.[47,54] During combination therapy with troglitazone and a sulfonylurea, increases in mean body weight ranging from ~0.5 to 6 kg have occurred.[73,77]

Hypoglycemia

Due to the mechanisms of action of these agents, hypoglycemia generally does not occur with metformin, acarbose, or troglitazone as sole agents.[18,21,57] Hypoglycemia with metformin[78] or troglitazone[77] is possible during concomitant use with other glucose-lowering agents, such as sulfonylureas, insulin, or ethanol.

Acarbose may increase the hypoglycemic potential of sulfonylurea therapy when used in combination.[79] Hypoglycemia occurring during acarbose therapy must be treated with glucose rather than sucrose because the mechanism of action of acarbose results in delayed gastrointestinal absorption of sucrose.

GLYCEMIC CONTROL WITH MONOTHERAPY

The sulfonylureas have been extensively used in clinical practice as first-line drug therapy in the management of type 2 diabetes, particularly in the United States, where they have, until recently, been the only oral hypoglycemic agents available.[19] Although sulfonylureas vary in potency, they are similar in efficacy.[14] Studies have reported decreases in basal and postprandial plasma glucose (PPPG) levels of ~3–5 mmol/L following 3–6 months of treatment with sulfonylureas.[80] Glycated hemoglobin has also been demonstrated to decrease by 20%.[80]

Three double-blind, randomized, placebo-controlled clinical studies have reported significant reductions ($P < 0.001$) in fasting plasma glucose concentrations (FPG) (22–26% of pretreatment levels) and glycated hemoglobin levels (12–17% of pretreatment levels) with metformin monotherapy.[75,81,82] Furthermore, metformin monotherapy is comparable to sulfonylurea monotherapy in maintaining glycemic control in studies of up to 3 years' duration.[22,26-29,66,67,83,84]

Metformin effectively controls hyperglycemia in both lean and overweight patients and in the elderly, and its use has often led to weight reductions.[28,29,63,66,75,85] As a result of this and its positive lipid effects, metformin may be beneficial in patients with mild to moderate hyperglycemia who are also dyslipidemic and/or prone to weight gain.

The clinical efficacy of acarbose monotherapy is more difficult to assess because of the current lack of published well-controlled studies. Most published trials to date have had small study populations (≤ 20 patients) and/or administered acarbose in doses exceeding 100 mg three times a day.[20,57] One large ($n = 100$) randomized,double-blind, placebo-controlled published study using 100 mg of acarbose 3 times daily demonstrated that the drug significantly ($P < 0.05$) improved glycemic control.60 Following 6 months of acarbose therapy, mean baseline PPPG, FPG, and glycosylated hemoglobin decreased by 25, 14, and 7%, respectively. Overall, there was a

nonsignificant trend for acarbose to be less effective than metformin or the sulfonylureas.[20]

Results from the majority of studies of troglitazone have been published only in abstract form. Based on reductions from baseline in fasting plasma or serum glucose and glycosylated hemoglobin, troglitazone appears to be less effective than metformin or the sulfonylureas. In a large (n = 328) double-blind study in type 2 diabetic patients, troglitazone 200–800 mg once daily for 3 months reduced fasting serum glucose by 8–13% from baseline levels.[54] There was no change from pretreatment levels in glycated hemoglobin levels with the 600 and 800 mg/day dosages, a slight increase (by 4% of pretreatment levels) with the 400 mg/day dosage, and a slight decrease (by 4%) with the 200 mg/day dosage.

The above study, however, demonstrated that troglitazone is superior to placebo. Compared with the values achieved with placebo, the values in troglitazone-treated patients (200-800 mg once daily) were significantly lower ($P < 0.01$) for glycated hemoglobin (by 7–13%) and fasting serum glucose levels (by 15–25%).[54] Similarly, a smaller (n = 93) double-blind, placebo-controlled study demonstrated significant ($P < 0.02$) reductions in FPG (by ~25%) and PPPG (by ~20%) with troglitazone 400 or 600 mg/day.[45]

Results of two separate comparative studies suggest that troglitazone monotherapy may be comparable to either sulfonylurea[48] or metformin[49] monotherapy, after 12 and 3 months of therapy, respectively. However, before definite conclusions can be made regarding the efficacy of troglitazone relative to the other two agents, these results need to be confirmed in other well-controlled comparative studies.

Repaglinide's initial doses range between 0.5 mg (naïve patients) and 2 mg before meals. The maximum dose is 4 mg before four meals. Patients should be instructed to omit the dose if the meal is to be omitted to avoid hypoglycemia. A fall of 1–2% in HbA_{1c} is to be anticipated. Since the drug's effect is greatest postprandially, it should be particularly effective in those with exaggerated postprandial values. Long-term side effects, if any, have yet to be determined.[23,50]

GLYCEMIC CONTROL WITH COMBINATION THERAPY

A major problem in the management of type 2 diabetes is that glycemic control (e.g ., maintenance of FPG $\leq$7.8 mmol/L [140 mg/dl]) with diet and/or drug treatment declines as the disease progresses. Various antidiabetic combination therapies have been established to overcome this and should be introduced as soon as diet or drug monotherapy fail.

Oral Combinations

Metformin Plus Sulfonylureas

Metformin and sulfonylureas have different mechanisms of action that work synergistically to alleviate hyperglycemia when the two agents are used in combination. There have been consistent reports of incremental decreases in FPG levels by 20% or more when metformin was added to existing sulfonylurea therapy in patients inadequately controlled by maximum doses of the sulfonylurea.[75,86-88]

Combination therapy with metformin and sulfonylureas is as effective as combined insulin/sulfonylurea therapy or insulin monotherapy in individuals presenting with treatment failure.[87,89-93] Consequently, the addition of metformin therapy may reduce the need to add insulin therapy when secondary failure with sulfonylurea drugs occurs.

Sulfonylureas Plus Acarbose
In contrast to combined therapy with metformin plus sulfonylureas, an acarbose-plus-sulfonylurea combination has an additive effect. As previously noted, data from published acarbose studies are limited by their small patient numbers (≤20 patients)[94,95] Combined therapy with acarbose plus sulfonylurea has consistently resulted in substantial decreases in PPPG and FPG levels.

Metformin Plus Acarbose
Data from a 1-year randomized, double-blind, placebo-controlled study evaluating the efficacy of adding acarbose to pre-existing therapy (including diet plus metformin) showed that the addition of acarbose resulted in significant decreases ($P < 0.001$) in mean PPPG levels for all treatment groups except placebo.[70] The PPPG decrease for metformin recipients was 18%. There was no significant difference in FPG levels between the acarbose-plus-metformin and metformin-plus-placebo groups. This study was conducted with doses of 300 mg/day and greater.

Troglitazone Plus Sulfonylureas
Troglitazone and sulfonylureas act by different mechanisms of action and produce a synergistic lowering of hyperglycemia when used in combination. In a double-blind, placebo-controlled study in 552 type 2 diabetic patients inadequately controlled by maximum doses of glyburide (12 mg/day), dose-dependent decreases from baseline in FPG of 14, 16, and 25% were observed following 1 year of concurrent therapy with troglitazone 200, 400, and 600 mg, respectively.[77] Concurrent administration of troglitazone 400 mg/day, given in a 200 mg twice-daily regimen, produces a reduction in FPG (15%) similar to that seen with once-daily 400 mg dosing.[73]

Metformin Plus Troglitazone
Concurrent administration of metformin (2,000 mg/day) and troglitazone (400 mg/day) demonstrated an additive antihyperglycemic effect in 28 type 2 diabetic patients with poor glycemic control at study entry (mean FPG 15.8 mmol/L [284 mg/dl]; HbA_{1c} 9.6%).[49] Most of these patients had secondary sulfonylurea failure. Combination therapy significantly reduced ($P < 0.001$) FPG (by 32–42%) and HbA_{1c} (by 13–16%), compared with the corresponding levels for these glycemic parameters achieved with either drug alone.

Metformin Plus Repaglinide
Moses and associates demonstrated that the combination of metformin and repaglinide was more effective than when either of the two drugs was used alone.[51] Initial dosing and monitoring should be the same as when either agent is used alone.

Combinations With Insulin
In the late stages of type 2 diabetes, secondary failure to oral drug therapy often occurs, and insulin eventually becomes necessary. However, type 2 diabetic patients often have such severe insulin resistance that effective glycemic control can only be achieved with large doses of insulin.[19,96] Combining insulin with an oral antidiabetic agent is a means of improving or maintaining glycemic control while reducing exogenous insulin requirements.

Sulfonylureas Plus Insulin
Insulin has traditionally been used alone or in combination with sulfonylureas. Numerous studies have shown that combined sulfonylurea-plus-insulin therapy results in a reduction in exogenous insulin requirement, but not all studies have shown combined therapy to be superior to insulin

alone in improving glycemic control.[97] However, it appears that improvements in glycemic control are achieved more consistently if insulin is added to ongoing sulfonylurea therapy at the time that secondary sulfonylurea failure occurs rather than if sulfonylurea is added to failed insulin monotherapy.[98]

In two small, double-blind, placebo-controlled studies ($n = 21$, $n = 30$), combined therapy with insulin (single injection in the evening/bedtime) plus either glyburide (10 mg/day) or glipizide (40 mg/day) was significantly superior ($P < 0.05$) to insulin alone in improving glycemic control.[99,100] In one of these studies, 10–16 weeks of combination therapy with glyburide plus insulin led to a FPG value that was 21% lower than the FPG value with insulin alone (5.9 mmol/L [106 mg/dl] vs. 7.5 mmol/L [135 mg/dL]; $P < 0.05$), and a significantly greater ($P < 0.05$) decline from baseline in glycosylated hemoglobin absolute value (1.3 vs. 0.8%).99 Patients receiving combined therapy required one-half the mean amount of insulin as those receiving insulin alone (50 vs. 101 U with insulin alone).

In 145 obese type 2 diabetic patients with secondary sulfonylurea failure, combination therapy with glimepiride (up to 16 mg/day) plus a single dinnertime injection of 70/30 insulin was as effective as insulin monotherapy in achieving a target FPG level of 7.8 mmol/L (140 mg/dl).[98] Combination therapy resulted in earlier improvement in glycemic control and a reduction in daily insulin dosage (by 29 U), compared with insulin alone.

Combined insulin-plus-sulfonylurea therapy appears to offer no advantage over insulin alone in reducing the tendency for weight gain or risk of hypoglycemia.[98-100] In the above studies, mean weight gains were 4.9 kg with insulin plus glyburide (vs. 3.3 kg with insulin alone)99 and 2–4.5 kg with insulin plus glipizide (vs. 0.6 kg with insulin alone).[100] The mean frequency of hypoglycemic episodes was slightly higher with combined therapy: 8.8 with glyburide plus insulin versus 6.9 with insulin alone[99] and 0.19 per patient per week with glipizide plus insulin versus 0.09 per patient per week with insulin alone.[100] Weight gain and hypoglycemic episodes were reported to be equivalent with glimepiride plus insulin versus insulin alone.[98]

In type 2 diabetic patients with poor glycemic control despite treatment with insulin alone, adding a sulfonylurea to the pre-existing insulin regimen improves glycemic control but may not be effective in achieving adequate glycemic control.[98]

Metformin Plus Insulin

Limited data are available on the use of metformin in combination with insulin. In two double-blind, placebo-controlled studies, plasma fasting insulin levels and daily exogenous insulin requirements were reduced following the addition of metformin to the insulin regimen.[101,102] The larger of these studies 101 involved 50 obese patients with poorly controlled type 2 diabetes despite $\geq$3 months of treatment with insulin at a mean daily dose of 90 U. The addition of metformin (850 mg twice daily) resulted in a significant improvement ($P < 0.05$ vs. baseline and vs. placebo) in glycemic control. After 6 months of combination therapy, there were significant reductions ($P < 0.05$) from baseline in mean glucose profile (by 34%) and in HbA_{1c} (by 16%, which represents a reduction in mean absolute value of 1.9%). The addition of metformin permitted a reduction in daily insulin dose of 24% (-21.6 U). No changes in body weight occurred during combination metformin-plus-insulin therapy.

Acarbose Plus Insulin
Data from a 1-year, randomized, double-blind, placebo-controlled study of combined acarbose-plus-insulin therapy showed a 15% reduction in mean PPPG levels compared with placebo.[70] Although the reduction was statistically significant ($P < 0.001$), the doses of acarbose used were in the 600 mg/day range.

Troglitazone Plus Insulin
Troglitazone is approved for use in combination with insulin in the treatment of type 2 diabetes patients who require insulin. In such patients, concomitant troglitazone improves glycemic control and generally enables a reduction in the daily dose of insulin.[77] In two double-blind, placebo-controlled studies, concomitant administration of troglitazone 400 or 600 mg/day for 6 months resulted in reductions from baseline in daily insulin dose of 58% and 42%, respectively, while glycemic control was improved or maintained.[77]

Some patients may be able to discontinue insulin. No reduction in insulin dose is recommended at the outset when prescribing troglitazone to poorly controlled, insulin-requiring type 2 diabetic patients. However, during concomitant troglitazone therapy, it has been recommended to decrease the dose of insulin by ~10–20% (the manufacturer recommends 10–25%), to reduce the risk of hypoglycemia when fasting and/or pre-meal glucose levels consistently drop below 6.7–7.8 mmol/L (120–140 mg/dl).[103]

SIDE-EFFECT PROFILES

Sulfonylureas

Sulfonylureas are generally well tolerated. The most common and also the most serious adverse event associated with these agents is hypoglycemia.[14,16,19] Severe sulfonylurea-induced hypoglycemia occurs with an estimated incidence of 0.19–2.5 episodes per 1,000 patient-years,[16] and the incidence is higher for the long-acting sulfonylureas, especially chlorpropamide (0.34/1,000 treatment-years) and glyburide (0.38/1,000 treatment-years).[19] In a 3-year study, hypoglycemic reactions occurred in ~13% and 27% of patients receiving chlorpropamide and glyburide, respectively.[29] The most important predisposing factors for sulfonylurea-induced hypoglycemia are increasing age and impaired renal function.[104,105]

Other side effects with sulfonylurea therapy are rare and include dermatological hypersensitivity, gastrointestinal discomfort, and vasomotor symptoms (most frequently reported with chlorpropamide).[19]

Metformin

The most common adverse effects of metformin are gastrointestinal symptoms,[16,18,52] which may be relieved by dosage reduction and rarely require discontinuation of treatment.[52,106] During long-term metformin administration, only 4.2% of patients discontinued therapy because of gastrointestinal side effects.[86]

Malabsorption of vitamin B12 and decreased folate absorption have been infrequently reported with long-term metformin therapy. Although there are no clinical manifestations of these effects, annual serum B12 measurements are recommended.[16,18,52,78] These decreases are rapidly reversible with vitamin B12 supplementation or discontinuation of metformin therapy. Only three

cases of megaloblastic anemia have been reported in the literature with metformin therapy.[107-109]

Lactic acidosis, a serious and potentially lethal metabolic condition, has occurred with all biguanides, but rarely with metformin. The mean incidence of lactic acidosis associated with metformin therapy is only about 0.03 cases per 1,000 patient-years.[18,104] Strict observance of contraindications and prescribing precautions substantially reduces this risk.[16,78]

Data from a retrospective study conducted in Sweden from 1977 to 1991 indicate that the reported incidence of metformin-associated lactic acidosis is low and is decreasing.[110] The incidence of lactic acidosis is lower than that of the equally serious sulfonylurea-induced hypoglycemia. A retrospective comparative risk study in Sweden reported that, between 1972 and mid-1981, the incidence of glyburide-induced hypoglycemic coma (0.19 per 1,000 patient-years of use) was significantly ($P = 0.036$) greater than the incidence of metformin-associated lactic acidosis (0.08 per 1,000 patient-years of use).[105] The risk of mortality from metformin-induced lactic acidosis is slightly lower than the mortality risk from glyburide-induced hypoglycemia (0.24 per 1,000 patient-years vs. 0.33 per 1,000 patient years, respectively).[105,111]

Acarbose

The most common adverse events associated with acarbose therapy are gastrointestinal disturbances, most frequently abdominal pain, diarrhea, and flatulence.[57] These events arise from the drug's mechanism of action and are related to the presence of undigested carbohydrate in the lower gastrointestinal tract. Several studies have demonstrated that these adverse events decrease as the duration of acarbose therapy increases. Moreover, the severity of the gastrointestinal disturbances may be reduced by decreasing the dose of medication and by good dietary habits.[20,57]

Elevations in serum transaminase levels may occur during acarbose therapy. In studies of up to 12 months' duration, treatment-emergent elevations of serum transaminases occurred in 15% of acarbose recipients compared with 7% of placebo recipients.[79] These elevations appear to be dose-related and are asymptomatic, reversible, more common in women, and in general not associated with other evidence of liver dysfunction.[79]

Troglitazone

Troglitazone is generally well tolerated.[77] The most serious adverse event reported with short- and long-term troglitazone therapy is idiosyncratic hepatocellular injury, although the incidence is rare. In most cases, hepatocellular injury manifested as reversible jaundice but led to hepatic failure or death in a few cases. Therefore, serum transaminase levels should be monitored at frequent intervals, especially during the first year of treatment and at the first signs of hepatic dysfunction (e.g., dark urine, fatigue, gastrointestinal disturbances). Troglitazone should be discontinued in patients developing jaundice or ALT levels >3 times the upper limit of normal.[77]

CONCLUSION

Oral antidiabetic compounds have an established role in the treatment of type 2 diabetes. Metformin is as effective as the sulfonylureas and superior to acarbose in controlling plasma glucose levels in patients with type 2 diabetes. Unconfirmed data suggest that troglitazone may be as effective as sulfonylureas and metformin. No conclusions can be drawn until further comparative data are available.

While the sulfonylureas, metformin, and troglitazone can cause serious adverse events, their incidences are low and can be minimized by strict adherence to the prescribing guidelines and close monitoring of treated patients.

Metformin has some advantages over sulfonylureas and acarbose, including the stabilization of body weight in patients in whom weight gain is a concern and the reduction of plasma lipid levels in individuals with hyperlipidemia. Troglitazone shares the weight advantage with metformin, but the two agents differ in their lipid effects. Total and LDL cholesterol are reduced by metformin but elevated by troglitazone; both agents reduce serum triglycerides.

Combination therapy using two antihyperglycemic agents with different but complementary mechanisms of action may improve glycemic control in patients with type 2 diabetes inadequately controlled by either agent alone. Both metformin and troglitazone are approved for use in combination with a sulfonylurea when failure of sulfonylurea monotherapy occurs.

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Return to Issue Contents

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EXHIBIT E

ORIGINAL CONTRIBUTION

Effect of Metformin and Rosiglitazone Combination Therapy in Patients With Type 2 Diabetes Mellitus

A Randomized Controlled Trial

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Context Most antidiabetic agents target only 1 of several underlying causes of diabetes. The complementary actions of the antidiabetic agents metformin hydrochloride and rosiglitazone maleate may maintain optimal glycemic control in patients with type 2 diabetes; therefore, their combined use may be indicated for patients whose diabetes is poorly controlled by metformin alone.

Objective To evaluate the efficacy of metformin-rosiglitazone therapy in patients whose type 2 diabetes is inadequately controlled with metformin alone.

Design Randomized, double-blind, placebo-controlled trial from April 1997 and March 1998.

Setting Thirty-six outpatient centers in the United States.

Patients Three hundred forty-eight patients aged 40 to 80 years with a mean fasting plasma glucose level of 12.0 mmol/L (216 mg/dL), a mean glycosylated hemoglobin level of 8.8%, and a mean body mass index of 30.1 kg/m^2 were randomized.

Interventions Patients were assigned to receive 2.5 g/d of metformin plus placebo (n = 116); 2.5 g/d of metformin plus 4 mg/d of rosiglitazone (n = 119); or 2.5 g/d of metformin and 8 mg/d of rosiglitazone (n = 113) for 26 weeks.

Main Outcome Measures Glycosylated hemoglobin levels, fasting plasma glucose levels, insulin sensitivity, and β-cell function, compared between baseline and week 26, by treatment group.

Results Glycosylated hemoglobin levels, fasting plasma glucose levels, insulin sensitivity, and β-cell function improved significantly with metformin-rosiglitazone therapy in a dose-dependent manner. The mean levels of glycosylated hemoglobin decreased by 1.0% in the 4 mg/d metformin-rosiglitazone group and by 1.2% in the 8 mg/d metformin-rosiglitazone group and fasting plasma glucose levels by 2.2 mmol/L (39.8 mg/dL) and 2.9 mmol/L (52.9 mg/dL) compared with the metformin-placebo group ($P<.001$ for all). Of patients receiving 8 mg/d of metformin-rosiglitazone, 28.1% achieved a glycosylated hemoglobin level of 7% or less. Dose-dependent increases in body weight and total and low-density lipoprotein cholesterol levels were observed ($P<.001$ for both rosiglitazone groups vs placebo). The proportion of patients reporting adverse experiences was comparable across all groups.

Conclusions Our data suggest that combination treatment with once-daily metformin-rosiglitazone improves glycemic control, insulin sensitivity, and β-cell function more effectively than treatment with metformin alone.

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TYPE 2 DIABETES IS CHARACTERized by decreased insulin secretion[1,2] and insulin sensitivity in liver, adipose tissue, and skeletal muscle. Together these abnormalities confound efforts to treat diabetes because most antidiabetic agents target only 1 underlying cause of the disease. Approximately 50% of patients treated with monotherapy require additional therapy to achieve target glycosylated hemoglobin (HbA_{1c}) levels 3 years after diagnosis.[3]

Rosiglitazone maleate, a member of the thiazolidinedione class of antidiabetic agents that was recently approved by the US Food and Drug Administration, targets insulin resistance by binding to the transcription factor peroxisome proliferator-activated receptor-γ, promoting synthesis of glucose transporters and activating adipocyte differentiation.[4-6] In contrast, metformin hydrochloride promotes glucose lowering by reducing hepatic glucose production and gluconeogenesis and by enhancing peripheral glucose uptake.[7-10]

Because metformin and rosiglitazone act through different mechanisms, their combined use may be indicated in patients whose disease is poorly controlled with a maintenance dose of metformin. This study evaluated the efficacy and safety of adding 4 mg/d and 8 mg/d of rosiglitazone maleate to maximal-dosage of metformin in patients with poorly controlled type 2 diabetes. Combined efficacy was assessed by comparing the level changes

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in HbA_{1c}, fasting plasma glucose (FPG), fructosamine, serum insulin, free fatty acids (FFA), lipids, lactate, and estimates of insulin sensitivity and β-cell function (BCF) between combined metformin-rosiglitazone treatment and metformin-placebo alone.[11]

METHODS

Study Subjects

To detect a 0.75% absolute difference in HbA_{1c} between treatment groups, 65 evaluable patients per group would be required to achieve a power of 95%. Planned enrollment was 280 patients (approximately 93 per group). Persons between the ages of 40 and 80 years with type 2 diabetes as defined by the National Diabetes Data Group[12] with FPG concentrations of between 7.8 and 16.7 mmol/L (140 and 300 mg/dL) at screening and during the placebo-maintenance period while taking 2.5 g/d of metformin were eligible. All patients demonstrated insulin secretory capacity as determined by a fasting C-peptide concentration of 0.27 nmol/L (0.8 ng/mL) or more at screening. Subjects were required to have a body mass index, calculated as weight in kilograms divided by the square of height in meters, of 22 to 38 and a weight change of no more than 10% between screening and baseline.

Patients were excluded if they had clinically significant renal or hepatic disease, angina, New York Heart Association Classification class III or IV cardiac insufficiency, symptomatic diabetic neuropathy, significant clinical abnormality on electrocardiogram, abnormal laboratory test results (blood chemistry, hematology, or urinalysis), use of chronic insulin therapy, participated in any rosiglitazone-related study, or used any investigational drug (excluding metformin) within 30 days of study (or 5 half-lives of the investigational drug, if longer than 30 days). Anorectic agents were discontinued at least 30 days before screening. Patients with hyperlipemia, elevated cholesterol or triglyceride levels, or lipid metabolism disorders were eligible; lipid-lowering agents were maintained at the same dosage level throughout the study.

Study Design

This multicenter, randomized, double-blind, placebo-controlled trial was conducted at 36 sites in the United States between April 1997 and March 1998. Before the study, patients discontinued all antihyperglycemic medications, with the exception of metformin. Metformin dose tolerability was determined during a 3-week period in which metformin was titrated to 2.5 g/d; afterward, patients entered a 4-week, single-blind metformin-placebo maintenance period with a weight-maintenance diet. During this maintenance period, only investigators were aware that patients were receiving the metformin-placebo treatment. Patients previously treated with metformin at 2.5 g/d proceeded directly to maintenance; thus, with the exception of metformin, patients refrained from medication for a minimum of 4 weeks and a maximum of 7 weeks.

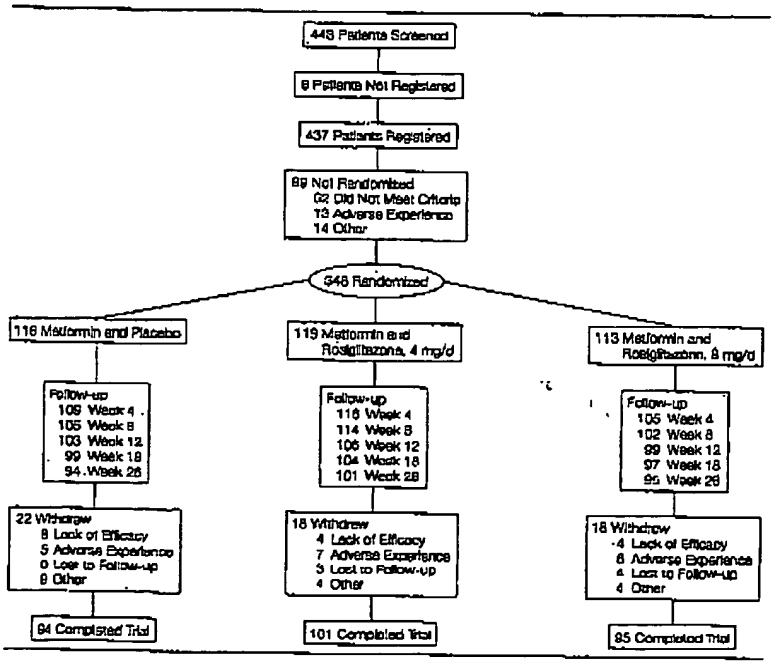
At the end of the maintenance period, patients with inadequate glycemic control (FPG concentration range, 7.7-16.7 mmol/L [140-300 mg/dL]) were randomly assigned (1:1:1 ratio) to receive double-blind metformin treatment in 1 of 3 combinations: placebo (control), 4 mg of rosiglitazone, or 8 mg of rosiglitazone once daily for 26 weeks. Randomization was computer generated with a fixed block size. No patient, investigator, or sponsor was aware of treatment allocation until study completion (FIGURE 1).

This study was conducted in accordance with the Declaration of Helsinki (as amended, 1989), Title 21 of the US Code of Federal Regulations, and Good Clinical Practice guidelines. The institutional review board at each center approved the protocol, and subjects provided informed consent before enrollment.

Efficacy and Safety Measurements

Laboratory measurements for efficacy and safety were performed by SmithKline Beecham Clinical Laboratories (Van Nuys,

Figure 1. Study Profile of Patients Randomized to Receive Metformin Hydrochloride Alone or With Rosiglitazone Maleate



Calif) on blood collected in the fasting state. Fasting plasma glucose concentrations, total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglyceride levels were measured by an Olympus analyzer (Olympus Clinical Instruments Division, Lake Success, NY); levels of HbA_{1c} were measured by the high-performance liquid chromatography method (Variant, Bio-Rad, Hercules, Calif); C-peptide by radioimmunoassay (Diagnostic Products, Los Angeles, Calif); insulin by radioimmunoassay (Linco Research Inc, St Charles, Mo); fructosamine by colorimetric analysis (RoTAG fructosamine assay, Roche Diagnostic Systems, Indianapolis, Ind); and FFA by enzymatic/colorimetric analysis (Wako Diagnostic, Richmond, Va) using a COBAS analyzer (Roche Diagnostic Systems). Low-density lipoprotein cholesterol (LDL-C) concentrations were estimated from total cholesterol and HDL-C determinations using the Friedewald calculation.[13] Lactate was measured by enzymatic spectrophotometric analysis using an Olympus analyzer (Olympus Clinical Instruments Division).

Estimates of insulin sensitivity determined by homeostasis model assessment (HOMA-S) and BCF (HOMA-B) were calculated using FPG and immunoreactive insulin values, or C-peptide levels. HOMA is a mathematical model based on glucose and insulin interaction in different organs, including the pancreas, liver, and peripheral tissues.[11] HOMA estimates of BCF and insulin sensitivity were calculated for each participant's FPG and insulin, or C-peptide levels, and expressed relative to values in a lean, nondiabetic reference population aged 18 to 25 years.[14-16] HOMA-S determinations of insulin sensitivity or insulin resistance have been validated by comparison with results of glucose clamp studies,[11,14] intravenous glucose tolerance tests,[11,15] and continuous infusion of glucose with model assessment.[15] The HOMA-B method has been validated by comparison with the intravenous glucose tolerance test and continuous infusion of glucose model assessment.[17] Application of HOMA has also been used in epidemiological studies.[18,19]

Safety monitoring included physical examination, vital sign assessment, weight measurement, electrocardiogram, adverse experience query, and laboratory tests.

Statistical Methods

The primary population for efficacy analysis was the intention-to-treat population, those with at least 1 value while receiving therapy (last observation was carried forward in the case of missing data or early withdrawals). Efficacy and safety parameters were measured at baseline and after 26 weeks of treatment. Safety parameters were assessed based on week 26 data (without the last observation carried forward).

Treatment groups were compared using analysis of covariance with terms for baseline, treatment, and center. The assumptions of the statistical model were tested before application. The Levene test of heterogeneity across treatments was applied at a significance level of $\alpha = .01$. If significant, the Shapiro-Wilk test of nonnormality ($\alpha = .01$) was examined. Parametric analysis or nonparametric analysis was used, depending on results of test assumptions. If prospectively defined assumptions for parametric analysis were not met, the Wilcoxon rank sum test was used. Pairwise comparisons to placebo used Dunnett multiple comparison procedure to maintain a 2-sided .05 significance level within each parameter. The statistical significance of the within-group change from baseline was tested by a paired t test or a signed rank test. Safety parameters, including clinical laboratory tests, vital signs, and body weight, were examined using 1-way analysis of variance. Statistical analyses were performed using statistical software (*SAS/STAT Software, Release 6.12*, SAS Institute Inc, Cary, NC).

RESULTS

Of 443 patients screened, 437 entered the duration and maintenance period and 348 were randomized to treatment (Figure 1). Most withdrawals were due to failing to meet inclusion criteria (69.7%). Baseline characteristics were similar among treatment groups (TABLE 1). Fifty-eight patients withdrew before completion of the double-blind phase: 22 from the placebo group and 18 from the 4-mg/d and 18 from the 8-mg/d rosiglitazone groups. Most participants withdrew because of adverse experiences or lack of efficacy (Figure 1).

Table 1. Baseline Demographic and Metabolic Characteristics of Randomized Patients (Intention-to-Treat Population)

Baseline Characteristics	Metformin Hydrochloride and Placebo (n = 113)	Metformin Hydrochloride and Rosiglitazone Maleate	
		4 mg/d (n = 116)	8 mg/d (n = 110)
Age, mean (SD), y	58.6 (9.2)	57.5 (10.5)	58.3 (8.8)
Sex, %			
Men	74.3	62.1	68.2
Women	25.7	37.9	31.8
Duration of diabetes, mean (SD), y	7.3 (5.7)	7.5 (6.3)	8.3 (6.3)
Prior treatment, %			
Diet and exercise only	4.4	6.0	4.5
Oral monotherapy	46.7	39.7	49.6
Oral combination therapy	46.9	54.3	51.8
Baseline HbA_{1c}, mean (SD), %*	8.6 (1.3)	8.9 (1.3)	8.9 (1.5)
Baseline fasting plasma glucose, mean (SD), mmol/L†	11.67 (2.91)	11.90 (3.17)	12.19 (3.05)
Body mass index, mean (SD), kg/m²	30.3 (4.4)	30.2 (4.2)	29.8 (3.9)
Race, %			
White	81.4	80.2	77.3
Black	3.5	6.9	10.0
Other	15.0	12.9	12.7

*HbA_{1c} indicates glycosylated hemoglobin.
†To convert from millimoles per liter to milligrams per deciliter divide by 0.0556.

Glycemic Control

The mean HbA_{1c} levels decreased significantly from baseline in a dose-dependent fashion in both rosiglitazone groups by 0.56% in the 4-mg/d and by 0.78% in the 8-mg/d rosiglitazone groups. But the control group experienced a significant increase in HbA_{1c} levels (0.45%) (FIGURE 2). Furthermore, both rosiglitazone groups had HbA_{1c} levels lower than those in the control group by 1.0% in the 4-mg/d and 1.2% in the 8-mg/d rosiglitazone groups. In contrast to results observed in the control group, the mean HbA_{1c} levels in the rosiglitazone groups decreased after week 4 and plateaued by week 18 (FIGURE 3). The percentage who achieved a 1.0% reduction in HbA_{1c} concentrations was 32.8% in the 4-mg/d and 37.3% in the 8-mg/d rosiglitazone groups and 7.1% in the control group.

Twenty-five (28.1%) of 89 patients taking 8 mg/d of rosiglitazone achieved the target HbA_{1c} control levels of 7.0%, and 51 patients (57.3%) in the same group achieved HbA_{1c} levels of 8.0%, or below the American Diabetes Association action point. Yet only 7.6% of the patients in the control group achieved HbA_{1c} levels of 7.0% and 35.9% achieved an HbA_{1c} level of 8.0%.

The mean baseline fructosamine levels of 341.73 µmol/L in the control group increased by 12.3 µmol/L. But in the rosiglitazone groups the levels decreased by 27.9 µmol/L from 340.9 µmol/L in the 4-mg/d group and by 36.8 µmol/L from 351.8 µmol/L in the 8-mg/d group (reference range, 200-278 µmol/L).

Although the mean FPG concentrations did not change significantly in the control group, they significantly decreased in a dose-dependent order from baseline in both rosiglitazone groups (1.8 mmol/L [−33.0 mg/dL], 4-mg/d rosiglitazone; −2.7 mmol/L [−48.4 mg/dL], 8-mg/d-rosiglitazone; $P<.0001$). The mean FPG concentrations in both rosiglitazone groups also had decreased compared with the control group (−2.2 mmol/L [−39.8 mg/dL], 4-mg/d rosiglitazone; −2.9 mmol/L [−52.9 mg/dL], 8-mg/d rosiglitazone; $P<.0001$) (FIGURE 4). Furthermore, FPG concentrations in both rosiglitazone groups decreased during the first 4 weeks, plateaued at 12 to 18 weeks, and remained stable thereafter (FIGURE 5). Nine patients (7.9%) in the control group, 25 (21.6%) in the 4-mg/d and 33 (30.0%) in the 8-mg/d rosiglitazone groups achieved FPG concentrations of less than 7.8 mmol/L (140 mg/dL).

Effects on Insulin Sensitivity and BCF

Adding rosiglitazone to maximum doses of metformin significantly increased HOMA-S values. The median baseline HOMA-S values ranged from 46.6 to 49.0 units. The HOMA-S values increased dose-dependently by 1.7 units in the 4-mg/d and by 3.8 units in the 8-mg/d rosiglitazone groups compared with the control group.

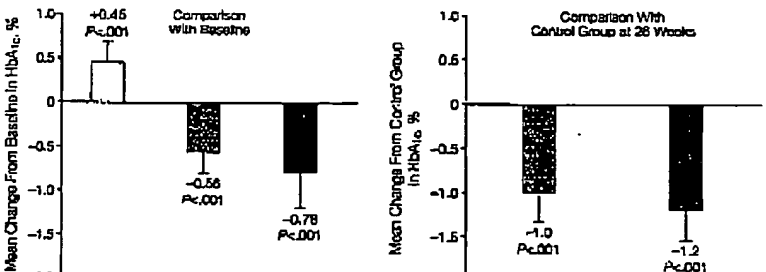
The metformin-rosiglitazone combination increased HOMA-B in a dose-dependent fashion. The median baseline HOMA-B values ranged from 32.5 to 35.8 units and were significantly increased by 10.3 to 13.7 units in the rosiglitazone groups compared with the control group.

Other Metabolic Effects

In the control group, the insulin value decreased by 11.05 pmol/L from a baseline of 118.56 pmol/L after treatment ($P = .03$) and in the 4-mg/d and 8-mg/d rosiglitazone groups the insulin values respectively decreased by 12.98 pmol/L from 124.55 pmol/L ($P = .01$) and by 31.07 pmol/L from 136.73 pmol/L ($P = .14$). The C-peptide values respectively decreased by 0.10 nmol/L from 0.93 nmol/L ($P<.001$), by 0.07 nmol/L from 0.92 nmol/L ($P = .01$), and by 0.12 nmol/L from 0.93 nmol/L ($P<.001$).

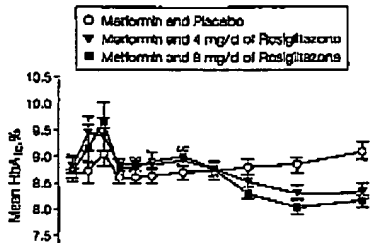
Mean total cholesterol-HDL-C, and LDL-C levels from baseline in both rosiglitazone groups achieved statistically significant increases in all treatment groups compared with the control group (TABLE 2). Total cholesterol–HDL-C ratios in the rosiglitazone groups were not significantly different from those in the control group.

Figure 2. Change in Glycosylated Hemoglobin (HbA_{1c}) Levels at Week 26 in Patients Taking Metformin Hydrochloride and Rosiglitazone Maleate Compared With Taking Metformin Alone



Error bars indicate 95% confidence interval.

Figure 3. Mean Change in Glycosylated Hemoglobin (HbA_{1c}) Levels Over Time in Patients Taking Metformin Hydrochloride Alone Compared With Patients Taking Metformin and Rosiglitazone Maleate Combined



Error bars indicate SE.

Changes in LDL-C levels were evaluated based on those at baseline. In that analysis, we identified 2 subgroups: those with levels lower than 3.37 mmol/L (<130 mg/dL) and those at that level or higher. We did not provide P values for any of the subgroups because the values were not large enough for statistical analyses and because the subgroups were not randomized, so significance could not be established. In the lower subgroup, the median baseline LDL-C value increased by 0.13 mmol/L (5 mg/dL) from 2.59 mmol/L (100 mg/dL) in 51 patients in the control group. In both rosiglitazone groups, the LDL-C values increased by 0.54-mmol/L (21 mg/dL) from a median baseline value of 2.69 mmol/L (104-mg/dL) in 57 patients taking 4-mg/d and from 2.64-mmol/L (102 mg/dL) in 60 patients taking 8-mg/d, resulting in medians that remained below 3.37 (<130 mg/dL) for all 3 treatment groups.

In the higher subgroup, the median baseline LDL-C value increased by 0.07 mmol/L (3 mg/dL) from 3.78-mmol/L (146 mg/dL) in 30 patients in the control group. In the rosiglitazone groups, the median baseline LDL-C value increased by 0.31 mmol/L (12 mg/dL) from 3.72 mmol/L (144-mg mg/dL) in 27 patients taking 4-mg/d and by 0.34-mmol/L (13 mg/dL) from 4.20 mmol/L (162 mg/dL) in 20 patients taking 8-mg/d.

Changes in triglyceride levels also were evaluated based on baseline values, using 2 subgroups: those with levels lower than 2.26 mmol/L (<200 mg/dL) and those with that level or higher. In the lower subgroup, the median baseline triglyceride values increased by 0.15 mmol/L (13 mg/dL) from 1.44-mmol/L (128-mg/dL) in 52 patients in the control group. In the rosiglitazone groups, the median baseline triglyceride value increased by 0.16 mmol/L (15 mg/dL) from 1.67 mmol/L (148-mg/dL) in 56 patients taking 4-mg/d and by 0.07 mmol/L (6 mg/dL) from 1.34-mmol/L (119 mg/dL) in 55 patients taking 8-mg/d. The treatment values in all groups remained less than 2.25 mmol/L (200 mg/dL).

In the higher subgroup, the median baseline triglyceride values decreased by 0.12 mmol/L (11 mg/dL) from 3.24-mmol/L (287 mg/dL) in 41 patients in the control group. In the rosiglitazone groups, the baseline median triglyceride value increased by 0.15 mmol/L (13 mg/dL) from 3.50 mmol/L (310 mg/dL) in 43 patients taking 4-mg/d and decreased by 0.72 mmol/L (64 mg/dL) from 3.16-mmol/L (280 mg/dL) in the 8-mg/d rosiglitazone group.

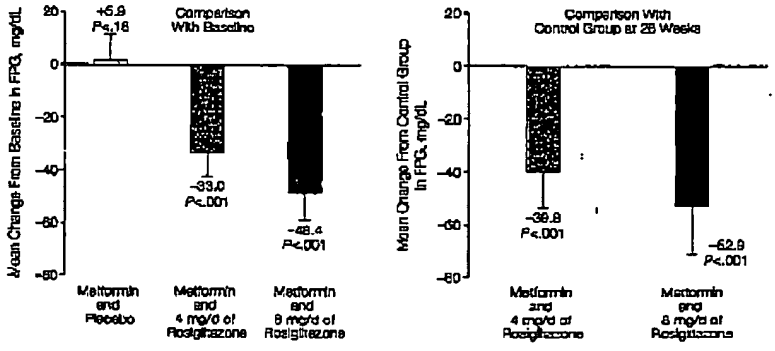
Mean fasting lactate levels decreased significantly in patients taking both dose levels of rosiglitazone compared with those in the control group (4-mg/d rosiglitazone, P = .012; 8-mg/d rosiglitazone, P = .002). Free fatty acids concentrations decreased significantly from baseline in both rosiglitazone groups. (TABLE 3).

Safety

The percentage of patients with at least 1 adverse event were comparable among each group (75.2%, 4-mg/d rosiglitazone; 78.2%, 8-mg/d rosiglitazone; 76.7%, control). The most frequently reported adverse events were upper respiratory tract infection, diarrhea, and headache. One death due to acute myocardial infarction occurred in the 4-mg/d rosiglitazone group but was judged to be unrelated to study medication. Serious nonfatal adverse events occurred in 5 (4.3%) of 116 patients in the control group and in 5 (4.2%) of 119 patients in the 4-mg/d and 5 (4.4%) of 113 patients in the 8-mg/d rosiglitazone groups, none considered related to study medication.

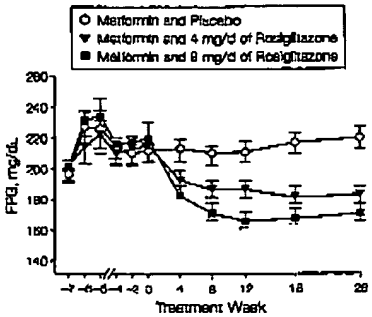
Symptomatic mild or moderate hypoglycemia was reported by 2 patients in the control group and by 3 patients in the 4-mg/d and by 5 patients in the 8-mg/d rosiglitazone groups. No patient required third-party interven-

Figure 4. Change in Fasting Plasma Glucose (FPG) Concentrations at Week 26 in Patients Taking Metformin Hydrochloride and Rosiglitazone Maleate Compared With Patients Taking Metformin Alone



To convert from milligrams per deciliter to millimoles per liter multiply by .0555. Error bars indicate 95% confidence interval.

Figure 5. Mean Fasting Plasma Glucose (FPG) Concentrations Over Time in Patients Taking Metformin Hydrochloride Alone Compared With Patients Taking Metformin and Rosiglitazone Maleate



To convert from milligrams per deciliter to millimoles per liter multiply by .0555. Error bars indicate SE.

tion or hospitalization, but the metformin dose was reduced from 2.5 g/d to 2.0 g/d in 2 patients. No one withdrew because of hypoglycemia, and there were no biochemically documented instances of FPG levels of less than 2.78 mmol (<50 mg/dL).

Both rosiglitazone groups experienced small but statistically significant decreases in hemoglobin and hematocrit levels, which occurred primarily during the first 12 to 18 weeks of treatment, after which values for both parameters increased slightly. The mean decreases in hemoglobin levels were −5.0 g/L in the 4-mg/d and −8.0 g/L in the 8-mg/d rosiglitazone groups ($P<.0001$ for both groups), and mean decreases in hematocrit were −1.8% in the 4-mg/d and −2.5% in the 8-mg/d rosiglitazone groups ($P<.0001$ for both groups). There were no significant changes in these parameters in the control group. One patient in each rosiglitazone group withdrew because of anemia, and 1 patient in the 4-mg/d rosiglitazone group with low hemoglobin and hematocrit levels was withdrawn from the study after week 8 because of evidence of gastrointestinal tract bleeding, considered by the investigator to be unrelated to the study medication.

There were no significant changes from baseline in vital signs or electrocardiogram parameters in the rosiglitazone groups compared with the control group. Although infrequent, edema was observed with greater frequency in the rosiglitazone groups (2.5%, 4-mg/d; 3.5%, 8-mg/d) than in the control group (0.9%). No one withdrew due to edema.

Those in the control group experienced a mean decrease in body mass of 1.2 kg from baseline, but those in the rosiglitazone groups experienced a mean body mass increase of 0.7 kg in the 4-mg/d and 1.9 kg in the 8-mg/d rosiglitazone groups ($P = .0001$ for both groups). There were no significant differences in waist-to-hip ratios among groups.

No one in the rosiglitazone groups experienced elevations of alanine aminotransferase (ALT) levels greater than 3 times the upper limit of the reference range. Mean changes in aspartate aminotransferase (AST), ALT, and total bilirubin levels were similar in all groups, with a slight decrease observed in mean ALT (−1.9 U/L, control; −1.9 U/L, 4-mg/d rosiglitazone; −3.4 U/L, 8-mg/d rosiglitazone). Mean alkaline phosphatase decreased in all groups (−3.5 U/L, control; −12.0 U/L, 4 mg/d rosiglitazone; −14.7 U/L, 8-mg/d rosiglitazone); the mean value for all groups was within the reference range. Two patients in the control group were

Table 2. Change in Lipid Parameters From Baseline at Week 26*

Lipid Parameter	Metformin Hydrochloride and Placebo	Metformin Hydrochloride and Rosiglitazone Maleate	
		4 mg/d	8 mg/d
Total cholesterol, mmol/L			
No. of patients	113	116	110
Baseline, mean (SD)	5.32 (1.02)	5.25 (0.92)	5.19 (1.22)
Week 26, mean (SD)	5.50 (1.03)	5.98 (1.05)	6.01 (1.41)
Mean (SD) change from baseline†	0.18 (0.61)	0.72 (0.74)	0.82 (1.07)
P value‡	.0018	<.0001	<.0001
Mean difference from placebo	...	0.53	0.60
P value§	...	<.0001	<.0001
HDL cholesterol, mmol/L			
No. of patients	112	116	110
Baseline, mean (SD)	1.14 (0.28)	1.18 (0.29)	1.20 (0.37)
Week 26, mean (SD)	1.20 (0.29)	1.32 (0.34)	1.36 (0.42)
Mean (SD) change from baseline†	0.06 (0.14)	0.13 (0.19)	0.16 (0.24)
P value‡	<.0001	<.0001	<.0001
Mean difference from placebo	...	0.08	0.10
P value§	...	.0002	.0002
Total cholesterol–HDL ratio			
No. of patients	112	116	110
Baseline, mean (SD)	4.86 (1.37)	4.62 (1.19)	4.56 (1.40)
Week 26, mean (SD)	4.79 (1.27)	4.80 (1.48)	4.77 (1.75)
Median change from baseline†	−0.015	0.115	0.130
P value‡	.73	.12	.18
Median difference from placebo	...	0.150	0.16
P value§	...	.13	.16
LDL cholesterol, mmol/L			
No. of patients	104	108	102
Baseline, mean (SD)	3.03 (0.88)	2.99 (0.78)	2.91 (0.84)
Week 26, mean (SD)	3.13 (0.97)	3.46 (0.86)	3.45 (1.04)
Mean (SD) change from baseline†	0.10 (0.44)	0.48 (0.58)	0.53 (0.76)
P value‡	.02	<.0001	<.0001
Mean difference from placebo	...	0.36	0.40
P value§	...	<.0001	<.0001
Triglycerides, mmol/L‖			
No. of patients	113	116	110
Baseline, mean (SD)	2.77 (2.19)	2.54 (1.56)	2.57 (2.07)
Week 26, mean (SD)	2.78 (1.79)	2.62 (1.57)	2.57 (1.87)
Mean (SD) change from baseline†	0.008 (1.32)	0.08 (1.35)	−0.003 (1.72)
P value‡	.95	.53	.98
Mean difference from placebo	...	−0.06	−0.10
P value§	...	.73	.56

*To convert cholesterol levels from millimoles per liter to milligrams per deciliter divide by 0.0259. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; and ellipses, not applicable. Data are rounded.
†Calculated only for patients with both a baseline and a week 26 value.
‡Significance level is .05.
§Significance level is .0259.
‖To convert triglyceride levels from millimoles per liter to milligrams per deciliter divide by 0.0113.

noted to have liver function tests for potential clinical concern (>3 times the upper limit of the reference range) while in treatment. Both completed the study with elevated transaminase values.

COMMENT

This is the first large, multicenter, clinical trial demonstrating the efficacy and safety of combined rosiglitazone and metformin treatment in patients with type 2 diabetes. The combination treatment of metformin and rosiglitazone significantly reduced HbA_{1c} and FPG concentrations, in a dose-ordered fashion compared with baseline and with metformin alone. Conversely, treatment with metformin was associated with significant increases in HbA_{1c} concentrations, indicating that these agents complement each other to achieve optimal glycemic control and confirming the clinical utility of metformin in combination with a thiazolidinedione drug.[20]

Consistent with the mechanisms of action of metformin and rosiglitazone, the reductions in FPG concentrations were proportionately smaller than those observed in HbA_{1c} concentrations. Maximum doses of metformin decrease hepatic gluconeogenesis, which principally affects FPG concentrations, whereas rosiglitazone enhances insulin sensitivity at the peripheral level and affects overall glucose disposal, including postprandial excursions. Because the relative contribution of postprandial glucose on glycemic control depends on the magnitude of FPG concentrations,[21] rosiglitazone may have an effect on postprandial hyperglycemia, as demonstrated directly in a rosiglitazone trial that showed significant improvements in fasting and postprandial glucose concentrations and excursions.[22]

The complementary actions of combined metformin and rosiglitazone is further supported by the effects of rosiglitazone on insulin sensitivity despite maximum doses of metformin. Rosiglitazone may provide added therapeutic value by reducing peripheral insulin resistance. While HOMA-S is an indirect method for determining insulin sensitivity, these results are consistent with glucose-clamp studies using other thiazolidinedione drugs.[23,24]

Table 3. Change in Free Fatty Acid Levels at Week 26 (Compared With Baseline and Metformin Hydrochloride and Placebo)*

	Free Fatty Acids, mg/dL		
		Metformin and Rosiglitazone Maleate	
	Metformin and Placebo (n = 113)	4 mg/d (n = 116)	8 mg/d (n = 110)
Baseline, mean (SD)	18.26 (7.75)	18.39 (7.56)	18.44 (8.00)
Week 26, mean (SD)	18.17 (8.06)	15.76 (8.05)	14.15 (8.13)
Change from baseline, mean (SD)†	−0.09 (7.56)	−2.61 (6.69)	−4.30 (7.88)
P value‡	.90	<.0001	<.0001
Mean difference from control	...	−2.52	−4.22
P value§	...	.0003	<.0001

*Ellipses indicate not applicable.
†Calculated only for patients with both a baseline and a week 26 value.
‡Significance level is .05.
§Significance level is .0259.

The improvements in HOMA-B with metformin-rosiglitazone treatment (not observed with metformin alone) were unexpected and introduce an important potential therapeutic benefit of rosiglitazone. Although the exact mechanism underlying this improvement remains to be determined, rosiglitazone-mediated reductions in glucotoxicity[25] and lipotoxicity secondary to elevated concentrations of circulating FFA or both[26,27] are candidate mechanisms by which rosiglitazone may improve BCF. The effects of rosiglitazone on BCF and insulin sensitivity are consistent with its effects on long-term glycemic control and suggest that it may possibly delay or prevent disease progression.

Despite significant increases in total cholesterol, HDL-C, and LDL-C with the metformin-rosiglitazone treatments, the total cholesterol–HDL-C ratio, which did not change significantly, may be a better predictor of cardiovascular outcome than either total cholesterol or HDL-C levels alone.[28-30] Since this study was not designed to assess long-term lipid effects, the long-term significance of these changes is unknown; however, patients with baseline plasma LDL-C levels lower than 3.37 mmol/L (<130 mg/dL) remained less than that level after therapy. No significant changes in triglyceride levels were noted in any treatment group, and segregation of patients into subgroups revealed nonsignificant increases in patients with baseline triglyceride levels lower than 2.26 mmol/L (<200 mg/dL). Among patients in the 8-mg/d rosiglitazone group whose baseline was higher than 2.26 mmol/L (>200 mg/dL), there was a significant statistical decrease observed (64 mg/dL). The clinical significance of lipid level changes may be minimal, because lipid-lowering therapy may be often administered to patients with diabetes irrespective of prior heart disease history.[31,32]

Elevated FFA may play a role in the development of insulin resistance, because it is associated with increased hepatic glucose output[33,34] and may contribute to β-cell dysfunction via a lipotoxic effect.[26,27] Elevated FFA has also been linked to endothelial dysfunction and hypertension[35,36] and enhanced platelet aggregation and coagulation,[37,38] which may increase cardiovascular risk. Therefore metformin-rosiglitazone treatment was significantly more effective in lowering FFA than the metformin alone.

The weight gain observed in those receiving metformin-rosiglitazone treatment may be attributed to increased adipocyte differentiation,[39,40] fluid retention,[39,41] or increased appetite.[42] Despite weight increases, no significant differences in waist-to-hip ratio among groups were observed, suggesting that rosiglitazone treatment leads to increased energy storage in subcutaneous adipose sites that are not associated with

increased cardiovascular risk.[43] The small decreases in hemoglobin and hematocrit levels associated with metformin-rosiglitazone therapy may relate to plasma volume expansion derived from fluid retention and hemodilution.[44]

Metformin-rosiglitazone therapy may be a safe alternative therapy to attain optimal glycemic control where monotherapy has failed because the statistically significant decreases in lactate levels associated with metformin-rosiglitazone treatment indicate that rosiglitazone may correct metabolic abnormalities beyond reducing hyperglycemia, and further suggest differing and complementary actions of metformin and rosiglitazone; and ALT elevations greater than 3 times the upper limit of the reference range were not observed in either of the rosiglitazone groups.

In summary, combination metformin-rosiglitazone treatment is effective and safe in reducing hyperglycemia in patients with type 2 diabetes. In patients whose fundamental abnormality is insulin resistance, such a combination raises the exciting possibility of treating diabetes by targeting the underlying cause of the disease, rather than the traditional approach of stimulating insulin secretion. Nearly 30% of patients taking the combination therapy achieved HbA_{1c} levels of 7% or less. This level of glycemic control is 3-fold greater than what was achieved among those taking metformin alone. Additional investigation is needed to determine whether this combination will alter the long-term risk of cardiovascular disease or delay disease progression.

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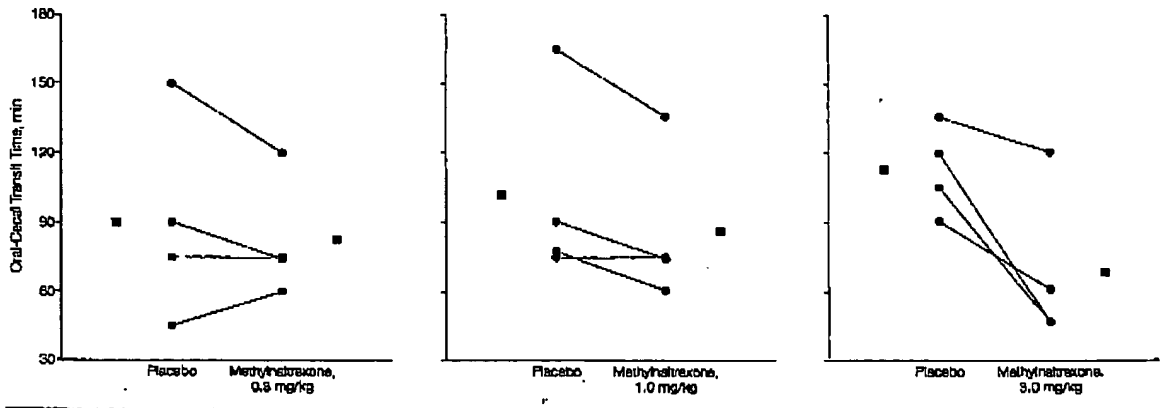
Figure. Dose-Related Changes in Oral-Cecal Transit Times



Changes in individual oral-cecal transit times of 12 patients receiving long-term methadone treatment after placebo and 3 oral methylnaltrexone doses (4 patients in each dose group). Squares represent mean values.

methylnaltrexone levels. Mean (SD [range]) peak plasma level for the other 4 patients (1 from the 1.0 mg/kg group and 3 from the 3.0 mg/kg group) was 17.8 (6.6 [10-26]) ng/mL.

Comment. Tertiary opioid antagonists, such as naloxone, cross the blood-brain barrier and reverse both the pain-relieving benefits and the adverse effects of opiates. Although oral naloxone may relieve opioid-induced constipation, the therapeutic index is very narrow,[5] and naloxone may induce opioid withdrawal symptoms. Many patients receiving opioid pain medications face a difficult choice between burdensome adverse effects or ineffective analgesia. Methylnaltrexone may allow for more aggressive use of opioid analgesics with fewer adverse effects. The low methylnaltrexone plasma levels observed in our study suggest that this charged compound acts directly in the gut. Oral methylnaltrexone has potential clinical utility in managing opioid-induced constipation with minimal adverse effects.

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CORRECTIONS

Incorrect Wording and Footnote Symbol: In The Rational Clinical Examination entitled "Does This Patient Have Carpal Tunnel Syndrome?" published in the June 21, 2000, issue of THE JOURNAL (2000;283:3110-3117), there was incorrect wording in the abstract. In the Conclusion paragraph on page 3110, the first sentence should have read, "Hand symptom diagrams, hypalgesia, and thumb abduction strength testing are helpful in establishing the electrodiagnosis of CTS." Also, in Table 2 on pages 3114 and 3115, the "No. of Hands" columns should include dagger symbols (†) instead of asterisks (*) down the column to indicate which studies used subjects instead of individual hands.

Incorrect Wording and Data Presentation and Omitted Acknowledgment: In the Original Contribution entitled "Effect of Metformin and Rosiglitazone Combination Therapy in Patients With Type 2 Diabetes Mellitus: A Randomized Controlled Trial" published in the April 5, 2000, issue of THE JOURNAL (2000;283:1695-1702), incorrect wording and incorrect data presentation were printed. On page 1695, in the "Results" section of the Abstract, the sentence that read "28.1% achieved a glycosylated hemoglobin of less than 7%" should have read "7% or less." On page 1698, the last sentence in the "Glycemic Control" section should have read "Nine patients (7.9%) in the control group, 25 (21.6%) in the 4-mg/d, and 33 (30.0%) in the 8-mg/d rosiglitazone groups achieved FPG concentrations of less than 7.8 mmol/L (140 mg/dL)." On page 1699 in the "Other Metabolic Effects" section, in the penultimate paragraph, which reports triglyceride findings, the phrase that read "In the rosiglitazone groups, the median baseline triglyceride value increased. . . by 0.07 mmol/L (6 mg/dL) from 1.34-mmol/L (119 mg/dL) in 56 patients taking 8-mg/d," should have read "decreased by 0.72 mmol/L (64 mg/dL) from 9.16 mmol/L (280 mg/dL) in 37 patients taking 8 mg/d." The last sentence of the penultimate paragraph of the "Other Metabolic Effects" section was repeated from the prior paragraph and should be deleted. In Table 2, the "Total cholesterol–HDL ratio" section should not have been converted to mmol/L. To calculate the proper ratio, divide the values in that section by 0.0259. In the footnote of Table 2, the cholesterol conversion factor should have read "0.0259." On page 1701 in the "Comment" section in the third column, the line that read "Among patients in the 8-mg/d rosiglitazone group. . . there was a significant statistical decrease observed (6.4-mg/dL)" should have read "(64 mg/dL)." In addition, Sylvia K. Chai, PhD, should have been included in the acknowledgment for her significant contributions to the preparation and review of the article.